PRACTICAL MANAGEMENT
PURE YEAST

PRACTICAL MANAGEMENT OF PURE YEAST

THE APPLICATION AND EXAMINATION OF BREWERY, DISTILLERY, AND WINE YEASTS

BY

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Third Edition

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PREFACE TO THIRD EDITION

Two editions of this book having been exhausted since its publication, the Laboratory has decided to publish a third edition, encouraged by the favourable reception accorded the book.

The book has been re-set throughout and its revision undertaken by Albert Hansen, Director, who, with his collaborators, has made all necessary additions and corrections in order that the book may represent all that is latest and best in practice.

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ALFRED JÖRGENSEN'S LABORATORY OF FERMENTOLOGY,
COPENHAGEN, 1936.

PREFACE TO FIRST EDITION

THE accompanying small book, making its appearance to supply a real want, is purely practical in its aims.

In regard to the constant control of the fermentations in the different branches of the industry, practical men have expressed a wish for a simple, comprehensive account of the way in which *samples*, sent to this Laboratory by men engaged in the fermentation industry, *are analysed*; also for a short, concise account of the leading features of *the treatment of pure culture yeast*; and for a short, complete review of the knowledge that has been acquired in this sphere of work by the constant co-operation of the Laboratory with the men who carry on the industry.

It is well known that it is easier *to put the problems before the analyst in proper form* when the limitations of analysis are known. Likewise it is easier for the practical man *to draw correct conclusions* from the information that is supplied to him, when he is acquainted with the principal features of the working methods of the Laboratory. A correct understanding of the principles on which analytical work is based is absolutely essential for *taking samples in the proper manner* in the brewery or distillery, etc., as the case may be.

Analysts have also desired a short description, of a

practical nature, of the outlines of *the elementary methods of analysis of alcohol-yeasts and of their pure culture*, and of the methods used here in the Analytical Laboratory and taught in the author's Laboratory for students.

It is evident that this short account cannot be regarded by analysts as a collection of receipts enabling them to carry through what work there may be to do. In order to employ these methods of analysis freely, and also to understand the principles on which they are based, it is necessary to have had practice in them under personal supervision, and to have studied a series of types of the Organisms of Fermentation.

This small hand-book, also, cannot do more than deal in a purely elementary manner with the most frequently occurring problems. The solution of the many special problems that arise demands at the outset more advanced study and greater experience.

The accompanying treatise will, then, be useful in making it easier for the practical man and the analyst to work together, as it reviews the most important methods of work, both practical and theoretical, employed in the control of yeast, each originating from a different basis.

This small book is, in a certain sense, a supplement to my book "Micro-organisms and Fermentation" (C. Griffin & Co., London). The latter book aims at affording a theoretical, historical, and critical account of the subject, accompanied by a description of a series of types of Fermentation Organisms. The practical side of the problem, therefore, both as regards the methods of analysis and pure

cultivation, could only be dealt with as regards its chief features.

Theoretical principles on which the methods are based, references to the literature of the subject, names of authors, etc., must be sought for in the above-named larger work.

ALFRED JÖRGENSEN.

THE LABORATORY OF PHYSIOLOGY AND
TECHNOLOGY OF FERMENTATIONS,
COPENHAGEN, 1901.

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PRACTICAL MANAGEMENT OF PURE YEAST

INTRODUCTION

WHILST carrying on fermentations in practice, one tries to discover the particular types of fermentative organisms that answer the necessary requirements under given conditions; and one attempts to find out the conditions that are necessary for the free and unhindered production of those properties that are of value, and one also naturally endeavours to guard against the competition of wild yeasts and bacteria.

It is no doubt evident that such proceedings *occupy some time before the results are clearly seen.* The micro-biological work that will engage our attention in the following pages concerns *the characteristic features* of the life-history of microscopical organisms. Life expresses itself in divers ways. The properties of organisms are manifold, and their condition of vigour changes unceasingly. The composition of the nutritive liquid (wort, mash, must, etc.) plays an important part. But in analytical work, as well as when making practical use of the results of such analysis, one must confine oneself to attending to *one* particular phase of this capability of exhibiting manifold properties; the particular piece of work must always exhibit one view only. Consequently, a definite series of such pieces of work must

often be carried out, each starting from a different standpoint, before one can possibly form any impartial opinion about the task before one. That one rapid experiment may be followed by a lucky result is the exception ; and it will not always reveal the real reason of the success met with in practice ; only when the real reason is discovered can one secure oneself from relapse. Only by *steady, systematic study of the conditions prevailing in practice* is it possible to understand the fluctuations, unavoidable in any Fermentation Industry, where living organisms have to be used.

THE ANALYSIS

THE ANALYSIS

THE examinations of the organisms of fermentation, undertaken at the present day in connexion with the different fermentation industries, are of two kinds—a distinction being made between, on the one hand, an analysis briefly carried out in order that information may be rapidly obtained as to the actual state of fermentations, and, on the other hand, a searching analysis carried out with the object of obtaining in detail definite information about the nature of the micro-organisms found to be present, and the special *rôle* which they play in practice.

When carrying out *rapid analysis* for purposes of general control, the question naturally enough suggests itself whether and to how large an extent a growth of foreign undesired organisms is present. An accurate analysis of this kind is usually sufficient for the purpose, as at the present day it is not at all a difficult matter to procure a reliable pure culture of a selected type of yeast. Such an analysis must be, then, carried out very quickly, and at short intervals, in order to be in a position to grapple with any irregularity as quickly as possible after its appearance. Experience has shown that a continued control of this nature is most essential, and it plays now a very important part in fermentation industries properly carried on.

A *detailed analysis* would be carried out when certain definite problems require solution. For example, when more detailed information is required regarding the germs

of different organisms found in the water or in the air: again, to ascertain if at a particular stage of the fermentation a particular type of foreign organism has obtained mastery, so that it is in practice important to obtain definite information about the character of the race of micro-organism concerned, and whence it came; or when "sickness" appears, and it is desired to trace out the evil to one of several possible causes, amongst others that a micro-organism may have crept in about which the rapid general analysis gave no special information; or again, perhaps it is desired to isolate *the different types of culture yeast* contained in the yeast mass in order to select a type of yeast from among them. Such investigations can be carried out with accuracy by experienced zymotechnologists, but require longer time than a general brief survey.

It is of the utmost importance to these different biological analyses that sufficiently large samples should be taken in the proper manner from fermenting vessels, casks, etc., to ensure an average sample of the organisms being obtained for examination. Later on, each single question that arises will be closely inquired into. It is now only necessary to note that for the biological analyses average samples of the liquid itself are not required. We must consider *in which portion of the liquid the living organisms are to be found* that are the object of our research. The distribution is not always the same. To take as a simple example the bottom fermentation tun, where fermentation proceeds slowly and evenly. We must remember that during the whole course of the fermentation secretions are brought up to the surface of the liquid, and often, as an examination will show, peculiar growths are found amongst these secretions—for example, film-forming types are found. When one is not careful to remove such film growths first, and then afterwards immerse the sample glass sufficiently deep in

the liquid, one obtains very incorrect information as to how the yeast *generally grows in the tun.*

In the case of bottom fermentations the samples for analysis should be withdrawn *a short time before racking.* Small bottles fitted with corks are employed for this purpose. The bottles are packed before use in white paper, and sterilised by being subjected to a heat of 150° C. (302° F.) for 2–3 hours. The corks are sterilised by being exposed to the action of formalin vapour in a closed flask. The man taking the sample takes the bottle out of the paper (his hands should be quite clean) just before using it, blows the head a little off the beer, plunges the bottle below the surface, opens it, and lets it fill itself, and then puts the cork in again before bringing it to the surface of the liquid and taking it out. The cork is then tightly fastened down with a piece of string, the bottle washed, and a label put on, with the number of the tun, gyle, and generation of the yeast written on it. In general a duplicate sample should be taken, amongst other things considering a later examination for Sarcina. Such samples may be allowed to stand several days, or be sent some distance away for examination, without deterioration.

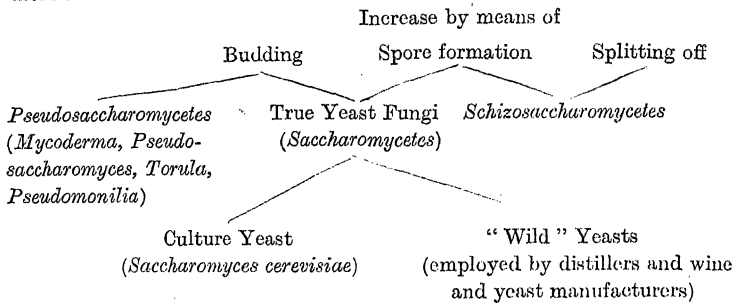
The liquid itself may be subjected to *micro-chemical* analysis in order to obtain information about the secretions that are present, and often very valuable information can be obtained from such an examination under the microscope —for example, information whether a wort does or does not contain unconverted starch.

I. ANALYSIS OF BREWERY YEAST

(*a*) A Simple Microscopical Examination

Before going into the analysis of yeast, the following table is given as a summary of the classification

of the *Saccharomycetes* after their modes of propagation :—



The information obtained by a simple microscopical examination of the yeast, whether an examination is made of the yeast suspended in the fermenting liquid or of the thick mass of yeast thrown up to the surface or down to the bottom of the tun, is for the most part only about the foreign bodies mixed with the mass of the yeast, namely, the bacteria, mould spores, and secretions thrown out of suspension from the liquid. As to the yeast itself, the following conclusions may be formed by this examination : namely, whether the majority of the cells *have the same appearance*, that is to say, if they show uniformly clear plasma, or whether vacuoles and small granulations have made their appearance, or whether the plasma is much granulated and the vacuoles have become irregular in size and shape ; or, again, whether the preparation under the microscope shows *irregular growth*—in that the above-named different growths of yeast cells are detected mixed in the yeast mass. Further, the cells may show differences as regards size, shape, and refractive powers. (Figs. 1, 2, 3 and 4.)

In by far the greater number of cases the cells of a normal growth of a yeast type will have the same appearance in regard to the above-mentioned characteristic features.

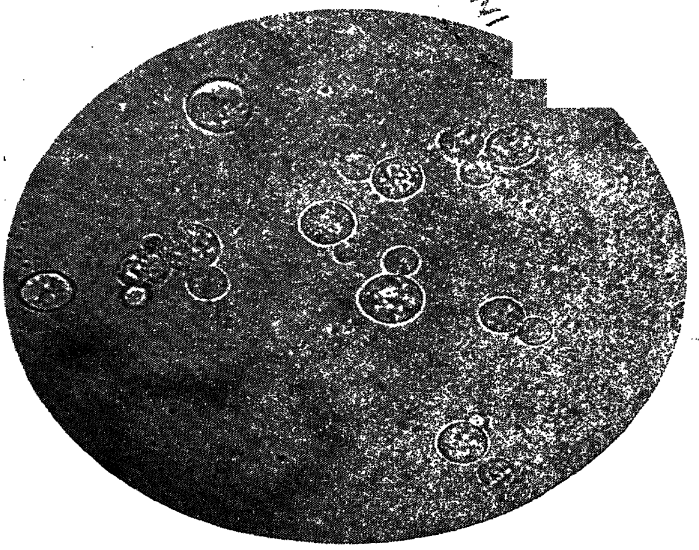


Fig. 1.—*Saccharomyces cerevisiae.* Top Yeast.

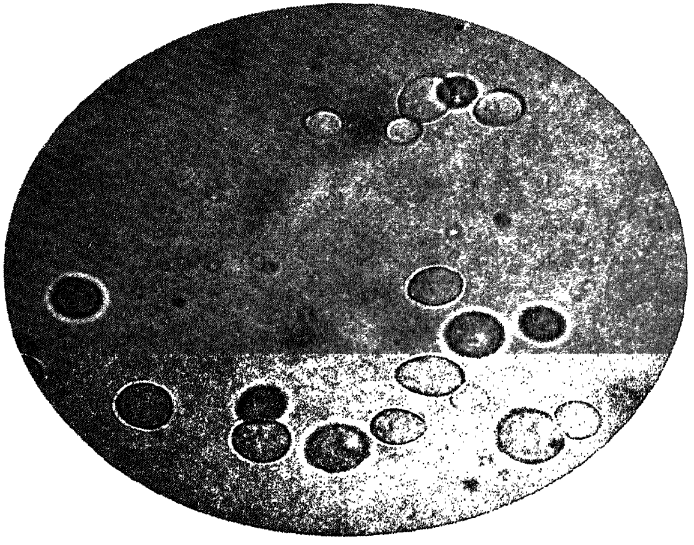


Fig. 2.—*Saccharomyces cerevisiae.* Bottom Yeast.

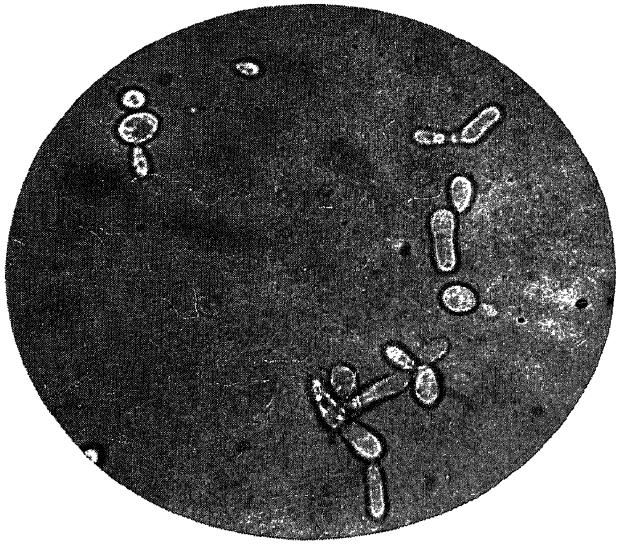


Fig. 3.—*Saccharomyces pastorianus.*

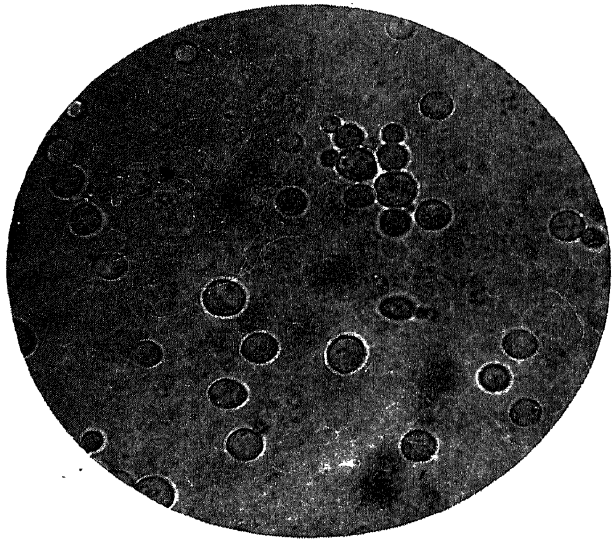


Fig. 4.—*Saccharomycetes ellipsoideus.*

Inexperienced workers will often have a difficulty in distinguishing between bacteria and secretions (albuminoid matter, hop resin, etc.). Beer contains, for example, secretions that can be confusingly like cocci forms of bacteria. Solvents (*e.g.* dilute soda solution, or acetic acid, or a mixture made up of ether and alcohol) are generally employed for the preparation of the microscopic slides in order to distinguish between secretions and bacteria. Such an examination must be undertaken with great care (strong solutions must never be used), and it must be clearly understood that an absolutely certain test of this nature is impossible. Sometimes, under certain conditions, the secretions are able to resist the action of the mediums employed; at other times the bacteria may be rendered so transparent that it is impossible to distinguish them. The best plan is to accustom the eye by examining and comparing several freshly prepared preparations, the contents of which are known, to work with accuracy in detecting the difference in the *refractive power and colour* of the matter in suspension, there being almost always a noticeable difference in these respects between secretions of albuminoid matter, etc., and bacteria.

The *very important question* whether the yeast mass contains "wild" yeasts or not cannot be decided by *a simple microscopical examination*, but recourse must be had to *spore cultures*.

(*b*) Analyses for the Rapid Detection of Wild Yeasts

Spore Cultures, using blocks of gypsum for growing them on, are of the greatest value, as it is thus possible to make an average estimate of the value of the sample by examining a large number of cells in a short time. This is accounted for by the fact that the difference between the spores of "culture" yeasts and wild yeasts is of so distinct

a character that one quickly notices it on placing the slide under the microscope.

Such an analysis only has its real value when the sample for examination is taken in the right manner and at the right time. It is not advisable to take a sample from the thick liquid yeast at the bottom of the tun, because the wild yeast cells found there are often either not able to form spores, or only form them late and to a slight extent.

It is, therefore, necessary to examine *the yeast cells suspended in the beer during fermentation.* In the *advanced stages of primary fermentation* the wild yeasts produce young cells, capable of rapidly forming spores.

A sample of the yeast suspended in the beer will, on analysis (made in the manner about to be described), give a clear indication as to the composition of the yeast used in the fermentation, and how the yeast which is settled out will develop, when it in its turn comes to be used for carrying on fermentation.

When the samples are to be examined they should be stood on one side for a few hours to settle. The greater part of the clear liquid is then carefully poured away, the sediment shaken up, and a few drops of it poured on to a block of gypsum. A better way is to pour off *all* the liquid and then shake up the sediment with a few drops of sterilised water, thus removing the greater part of the unfermented extract from the yeast.

The rest of the sediment is used for a microscopical examination, as already described, and for the biological analysis for detection of Sarcina (see Section *c*).

The cultures on blocks of gypsum (Fig. 5) (plaster of Paris) are set up as follows: A block of gypsum,* in shape like a cone with the top cut off, is placed in a round shallow

* To make a gypsum block, 2 parts of powdered gypsum are mixed with ¾ part of water and the mixture poured into a tin mould.

glass dish, fitted with a loose lid. The glass containing the block of gypsum is packed in paper and subjected twice to 110° C. (230° F.) for 1 hour, with an interval of 24 hours, to ensure sterilisation.

When the liquid yeast is to be poured from the bottle on to the block, the outside of the bottle is first carefully cleaned, and then the gas flame of a Bunsen burner passed carefully over it; next the glass cover is raised partially, and the mouth of the opened bottle is just put under the cover and a few drops of the liquid yeast poured on to different parts of the surface of the block of gypsum.

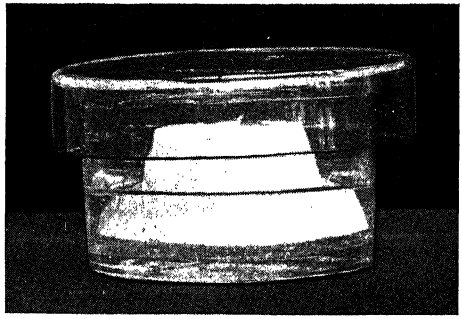


Fig. 5.—Glass Dish, with Gypsum Block.

Then sterilised water is poured round between the block and the glass until the level of the water has risen, say, half way up the block.

The cultures are now placed on one side at a constant temperature of 25° C. (77° F.), or sometimes 15° C. (60° F.), and the yeast cells will multiply, partly by budding and partly by building up spores internally.

When subjected to a temperature of 25° C. (77° F.), the wild yeast will have formed spores after forty hours; the culture yeasts that require a longer period than forty hours to develop their spores can, therefore, be distinguished

from the wild yeasts. Other types of culture yeasts, that develop their spores as rapidly at 25° C. (77° F.) as the wild yeasts, must be examined after being subjected to a temperature of 15° C. (60° F.) ; at such a temperature the wild yeasts will have developed spores after three days, whereas culture yeasts will not have done so till the fourth day or even later.

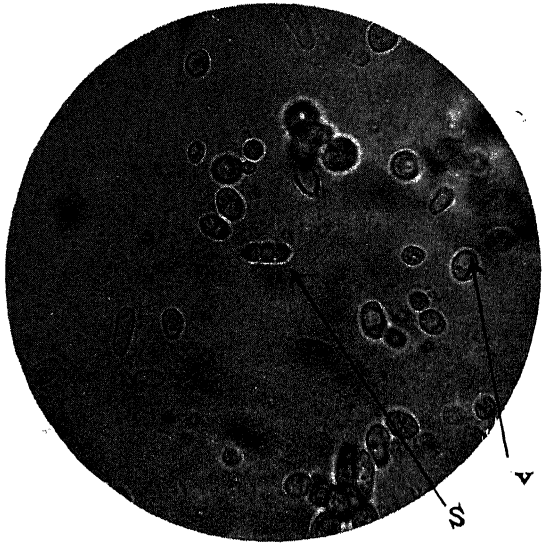


Fig. 6.—*Saccharomyces pastorianus*, with spores.
V = Vacuole. S = Spores.

A good method of distinguishing between the two groups of yeast is the *appearance of the spores.* A brewery bottom-fermentation yeast develops large spores ; their contents are not uniform, as granulations or vacuoles may be present ; they are all surrounded by a distinct thin wall. The wild yeasts produce small spores (Fig. 6) which under normal conditions of development (if the sample was taken in the right manner) show the contents of the spores to be clear and uniform, whilst the wall is indistinct. An

examination, having only this object in view, can quite well be carried out after *developing spore cultures under conditions of ordinary room temperature.*

In the examination of a single slide only, prepared for the microscope, thousands of cells present themselves to view. An accurate calculation as to the *extent* of the infection, by counting the cells, is plainly impossible. Such an attempt is also quite unnecessary in practice. All that is required is *definite information* as to whether the infection is serious or not.

When analysing *brewery top-fermentation yeast,* a microscopical examination of the yeast mass, or of the beer itself, is carried out for the detection of bacteria, etc., or it is tested for wild yeast infection.

No general rule can be laid down as to *when* the samples should be taken from the fermenting rounds in order to ascertain the amount of wild yeast infection, as the top fermentations are carried on in such different ways in different countries. The analyst must, after he has become thoroughly familiarised with the method of fermentation practised in the brewery concerned, withdraw several samples from near the surface of the fermenting wort, or from the heads themselves thrown up during the different stages of the fermentation; and by comparing his results ascertain which sample is best suited for this analysis. Here also, however, the following rule may be laid down—the best stage, in point of time, is reached when the change from the vigorous primary fermentation to the quiet after-fermentation takes place: this stage does not, however, always coincide with the racking of the beer.

Analyses for detection of wild yeast are conducted according to the method already described. In some cases it is difficult to distinguish between the two types of spores. When it is required to distinguish the spores

by noting the length of time required for the spores to form, it is often necessary to develop the gypsum block cultures at lower temperatures—for example, 10°–12° C. (50°–53° F.)—because some types of top yeast develop spores (Fig. 8) as rapidly as the wild yeasts if higher temperatures are employed. In the case where a small quantity of wild yeast is supposed to be present, a few drops of the yeast paste are transferred to sterile wort containing 4 per cent. tartaric acid and placed at 25° C. for 24 hours. In this manner the culture yeast is suppressed and the wild yeast favoured. After being recultivated twice at 24 hour intervals, ordinary gypsum block cultures are prepared from the resulting yeast and kept at 25° and 15° C.

(*c*) Analyses for Bacteria

The bacteria generally found in beer are—

(1) Acid-forming bacteria (lactic and acetic acid forming bacteria).

(2) Sarcina.

(3) Certain putrefactive bacteria which in the brewery are denominated as belonging to the *bacterium termo* group.

Bacteria may be found in bottom as well as in top yeasts, but are mostly found in the latter, and on account of the higher temperature during the fermentation, the lactic and acetic acid forming bacteria are often involved.

The *acid producing bacteria* can be distinguished either directly in the yeast with the microscope or by special cultivation.

It is not possible to distinguish with the microscope between the different types of acid-producing bacteria that are found in the yeast, or to distinguish them from other species of bacteria. The acetic bacteria do not, as

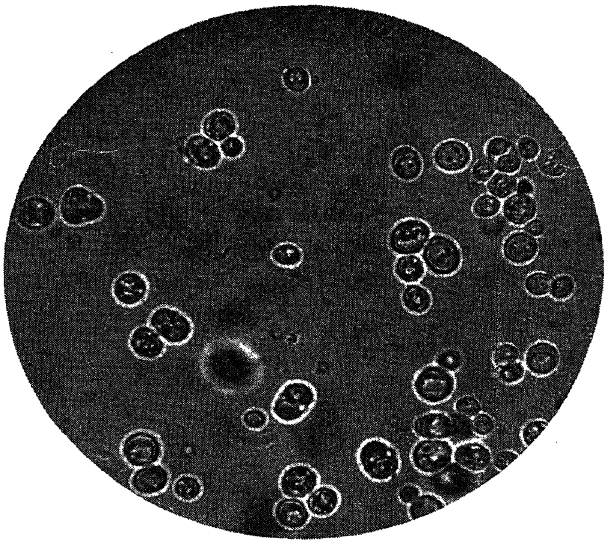


Fig. 7.—*Saccharomyces ellipsoideus,* with spores.

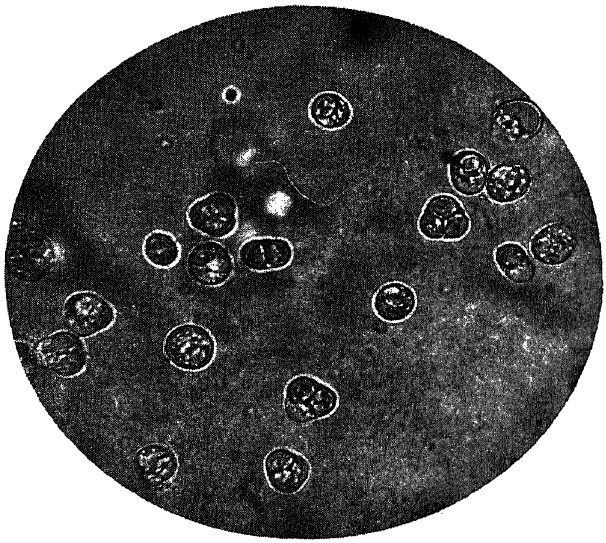


Fig. 8.—*Saccharomyces cerevisiae.* Top yeast with spores.

a rule, whilst fermentation is proceeding, become united together in the well-known chain form, and only a few cells exhibit distinct irregular swollen bodies.

Of the cultivation methods we shall here mention plate cultures on dextrose-gelatine with calcium carbonate. By means of this method it is possible not only to isolate the different bacteria, but also to get an idea about the proportion of the bacteria to the yeast cells.

Dextrose-gelatine is prepared as follows: 100 c.c. yeast water, 10 grms. lactose, 20 grms. dextrose, 5 grms. peptone, and tap water to 1,000 grms. The solution is boiled and filtered, 50 grms. precipitated calcium carbonate ($CaCO_3$) added and such quantity of gelatine that the medium contains 10 per cent. of it. The dissolution of the gelatine and the sterilisation of the medium should be done as described on page 55 (but without using any white of egg for clearing).

Dextrose-gelatine is melted by a temperature of about 28° C. (82° F.), and a few drops of the yeast—an average sample diluted in water—are carefully mixed with the melted medium. It is preferable to make 2 or 3 dilutions from the yeast by adding a few drops of yeast paste to 10 c.c. sterile water and add a few drops of this dilution to another 10 c.c. water. Plates are poured from the original paste as well as from the dilutions. These are plated out into Petri dishes. After exposure for some days at a favourable temperature (below 25° C. or 77° F.—because the gelatine will be melted at higher temperature), the colonies of lactic acid bacteria will have developed sufficiently for recognition. They are distinguished from any colonies of other species of bacteria by being surrounded with a transparent ring. This transparent ring is the result of the particles of chalk, close to the colonies, having been dissolved by the acid secreted by the bacteria. A micro-

scopical examination of some of the other colonies will reveal the presence of acetic acid bacteria, because they will now be found in the typical "chains." Some colonies of acetic acid bacteria also have a transparent ring or have a bluish opalescent surface.

Examination for Sarcina is carried out by pouring alkaline yeast water (*p*H 8–8·2) over the sediment in the sample bottles. A few drops of strong alcohol are added in order to suppress the development of other bacteria, and a few drops of autolyzed yeast (for preparation of yeast water and autolyzed yeast, see page 54).

The sample bottles, which should be filled completely, are placed, corks tied down, in the thermostat at 25° C. for 72 hours. If Sarcina are present they will develop sufficiently during this time in order to be revealed by a microscopical examination of the sediment.

Termo bacteria, consisting of short ovate rods, are often seen by a direct microscopical examination of the yeast, but these bacteria are also found in the afore-mentioned dextrose-gelatine culture. They may further be seen in the alkaline yeast water.

When the analysis of the yeast of bottom fermentation or top fermentation shows that the infection in the tun is really serious, it is well to remember that to throw away the yeast and to introduce pure yeast is only a half-measure; the first thing to be done is to *thoroughly disinfect the tun.* As a rule, a thorough cleaning of such a tun can be effected with bisulphite of lime, which must not be cleaned off when the tun is refilled, but be left on. If the tun shows distinct faults, such as cracks, small depressions, or a rough inside surface, a stronger and more penetrative disinfectant must be employed. As the most efficient disinfectant it is recommendable to use a dilution of ammonium fluoride (3–5 per cent.) or of formalin (1·5 per cent. = about 0·5

per cent. of formaldehyde); but the vessel must then be carefully washed with water.

If the contamination is very bad, the tun must then of course be thoroughly cleaned by mechanical means. Such an undertaking is carried out far too seldom in many of the fermentation industries.

(*d*) Analysis of the Growth of Bottom-Fermentation and Top-Fermentation Yeast at rather Advanced Stages of the Fermentation. Old Growths

Advanced growths of yeast are found, for example, in the sediment developed in the beer left standing in sampling bottles; in the deposit in lager casks; in the pitching yeast left undisturbed for a short or long time, as the case may be; in yeast pressed for exportation; in samples of yeast sent in filter-papers for examination from breweries, distilleries, etc., at a distance from the laboratories.

A microscopical examination of such samples will often reveal the presence of bacteria and moulds. Both in cask sediment and in thick-fluid yeast, and in pressed yeast, a large amount of fine secreted matter is found present. The analyst must make a most careful examination in order to distinguish the bacteria from these secreted matters.

Under these conditions the yeast will consist either of only exhausted cells, or a mixture of old and new cells.

The analytical examination to be made depends upon the information required.

If some of the cells are *budding*, and it is thought desirable to have some information as to *the nature of these cells*, whether they are culture or wild yeast cells, it is only necessary to develop ordinary spore cultures by

transferring some of the yeast at once to blocks of gypsum.

If, on the other hand, information is desired about the *different types of yeast present in the yeast mass generally*—for example, the proportion of culture and wild yeasts present, if one or more races of culture yeast are present, and so on—it is necessary to obtain a development of the whole growth by encouraging the budding of the exhausted cells. The quickest way is to introduce an average sample of the yeast into a Pasteur flask containing sterilised aerated wort, and let it grow there. It would be of advantage if the development of the yeast cells of the different types took place to an equal extent, so that spore cultures on blocks of gypsum would enable conclusions of a sufficiently practical nature to be formed regarding the ratio of culture yeast to wild yeast in the original yeast mass.

Such information is not, however, reliable. As development takes place in the flasks, the different types may again enter into competition, and the weaker cells in the mixture will be suppressed by the stronger; also the conditions governing the development of the cells in the flask will be more favourable to some types and less favourable to others than the conditions governing the growth of the yeast mass in the brewery or distillery from which it came.

If the yeast mass is treated in the manner just described, before spore cultures are set up, information is then only obtainable as to the type or types of yeast that will develop most vigorously in the flasks. If, for example, a development of wild yeast is noticed in a sample of yeast taken from deposit in cask, this development will usually continue to a very marked extent in the flask, if the growth of the yeast is allowed to continue to the fullest extent.

Definite information about the *proportion* in which the different types were present in the original sample is consequently only obtainable when it is possible to exclude competition between the types during the development of the vegetation.

If what is wanted is simply an *approximate estimate* for practical purposes of *the divers yeasts*, the materials must be prepared for such research-work by sowing a comparatively weak dilution of the yeast in wort in a series of Freudenreich flasks (Fig. 9, ii).

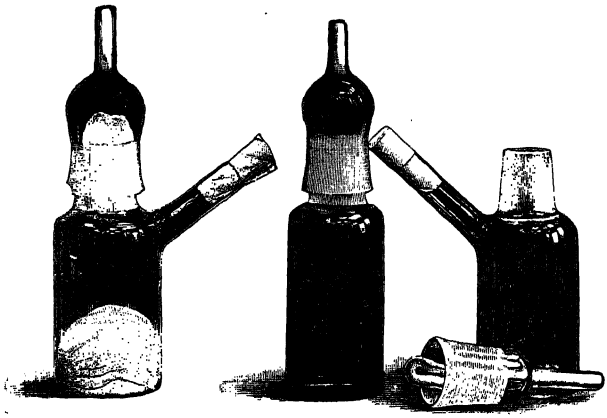


Fig. 9.—Flasks in which yeast cultures (i) can be sent through the post, or (ii) in which cultures may be developed, or (iii) preserved.

For this purpose a drop of the thick yeast is placed in a Freudenreich flask containing about 7–10 c.c. of sterilised water, and an estimate made of the number of cells contained in a little drop of the dilute solution. This is determined with the assistance of an ordinary cover slip, with small squares ruled out on the middle of it—twenty-five in number. An average sample of the solution is laid on the cover slip with the aid of a platinum loop, the slip is fastened to a glass ring (moist chamber), and the cells

are then easily counted under the microscope, the fine lines being of assistance in the process. The solution is now diluted by adding more sterilised water, until a drop, spread on the cover slip with the aid of the platinum loop, contains from 10 to 20 cells. Each of the 20 to 40 Freudenreich flasks is next infected with the aid of the platinum loop with a drop of the solution, and the flasks should afterwards be thoroughly shaken for some time, in order to make as sure as possible that the cells are well separated, and that each cell will sink independently to the bottom of the flask. The flasks are then allowed to stand at room temperature, and each of the independent cells lying on the bottom of the flask will develop a colony, appearing like a fleck against the glass. If the flasks are allowed to stand for some few days undisturbed, it will be noticed that each small colony will grow, and remain isolated, so to speak, from other similar small colonies in the flask; the exhausted cells have, therefore, time enough and sufficient space to produce young cells, instead of being suppressed through being in contact with those that are more vigorous, and are thus, in accord with our wishes, able to produce spores if spore cultures are carried out. The flask is thus representative of the ideal: each individual has free scope to grow and develop, and to assert itself without let or hindrance.

When the flask cultures are from four to five days old, having been kept at room temperature, the development of colonies will be sufficiently advanced. The growth in each flask may then be employed separately for spore cultures, or the contents of several flasks can be mixed up together. If the work up to this point has been carried out with good average samples, the final results of the research will represent for us the general proportions in which the different types of yeast are present under

practical conditions, and reliable conclusions can be drawn with regard to the conditions prevailing in practice.

To ascertain the different species of bacteria to be met with in such old cultures of yeast, the research must be conducted with cultures on gelatine, using as the cultivating medium one specially favourable to bacterial growth.

(*e*) Detailed Analysis

If information is required about the *characteristics* of the different *yeast types* found in mixed yeast—for example, about their behaviour in the liquid used in practice, or the temperatures most favourable to their development, and so on—the separation of the cells must be carried out so thoroughly that it is possible to work with vegetations grown from a single cell. The dilution process is carried out to such a degree that only one cell is found in every two or three drops; and these drops are then sown—one drop into each flask—in a large number of flasks containing a suitable medium for the development of the yeast cells. Some of the flasks will develop growths descending from a single cell. The flasks containing yeast colonies can be recognised by the small yeast "spots" settled on the bottom of the flasks, as each cell develops a small well-defined colony.

The methods of analysis described in the last section (weak dilution) will give some idea of the growth of the yeast on a larger scale; the above-described (strong dilution), going more into detail, will bring out further points of difference. A selection is made of all the flasks, and absolutely pure cultures grown, in moist chambers, from these selected colonies (see section describing Pure Culture). The different cultures are compared botanically and biologically, one with the other (see description of species in the author's "Micro-organisms

and Fermentation," 5th edition, published by C. Griffin & Co., London). An important test for the comparison of different yeast types is the film which yeasts spread little by little over the surface of the wort. A microscopical examination can reveal distinct points of difference in the different types in this respect; some develop a distinct branching mycelium, throwing out conidia.

Sometimes yeast cells that will not produce spores can be separated from such old growths, although from the appearance of the contents of the cells one would suppose that one was examining a yeast type that could produce spores. Sometimes, not always, the culture can be forced to develop spores by growing it for several generations in dextrose yeast water. That such old cultures lose the power of producing spores has been proved in the laboratory in the case of pure cultures kept for years. By repeated cultivation in sterile wort, not a few types of yeast have shown a marked falling off in their power of developing spores, and in some cases the power has been lost entirely. In such cases it is not always possible to restore this power, either by cultivation in dextrose solution or in any other way.

When a number of really different types or races of yeast have been secured by the methods just described, sufficient material has been obtained to consider the problem before us—the importance of these different types for fermentations in practice.

II. ANALYSIS OF THE YEAST IN DISTILLERIES AND YEAST FACTORIES

This analysis is in certain respects not quite so simple, and greater difficulties have to be encountered than when brewery yeast is the subject of analysis, as the process of manufacture is more complicated. This is accounted

for by the fact that because the mash and the wort are not boiled, many of the germs present, particularly bacteria, are not killed, but only somewhat crippled; it is, therefore, necessary to be prepared with different means at one's disposal to suppress the growth of these foreign germs, both during mashing and preparation of wort in air-yeast manufactories and also during fermentation. There is, therefore, much to be done in this respect, and a properly conducted micro-biological control is of the greatest value.

Temperature plays a very important part in regard to the amount of infection. If sufficient care is not taken to secure certain fixed temperatures throughout the mash or wort, foreign germs can easily gain the upper hand during fermentation.

A microscopical examination of the yeast taken from these rapid vigorous fermentations will be able to give useful conclusions. It is necessary that the yeast, whether it is the yeast grown in the acid wort and to be used for pitching, or the yeast skimmed from the tuns for the yeast press, or the yeast produced in the air-grown yeast factories, should be uniform and well grown; that is to say, the yeast must not be a mixture of full-grown ripe cells and cells that have produced only small buds, or contain a high proportion of granulated and exhausted cells.

Whether or not the pitching yeast grown in the acid wash is able vigorously to ferment the wash or wort in the large tun depends partly on the type of yeast selected, and partly on the concentration of the mash or wort and the degree of acidity; further, upon the pitching temperature, that is, the scale of temperatures at which the fermentation of the acid mash takes place, and also upon the final degree of attenuation. Experiments with

the selected race of yeast may give useful indications as to the point to which fermentation in acid mash is to be carried. Valuable information can also be obtained from the phenomena shown by fermentation in principal mash ; further, from the way in which fermentation sets in, or also from the degree of warming and attenuation.

The microscopical appearance of the pressed yeast, which is often used in many factories or distilleries as a pitching yeast, is a useful guide. In a normal yeast of this description the cells contain vacuoles comparatively large, and in the plasma single fine granulations. The presence of cells much granulated, and containing irregular or numerous small vacuoles, is, as a rule, an indication of the yeast being weak.

When a single race of pure selected yeast is employed, wild yeasts will develop only with difficulty during these short vigorous fermentations. If it should be necessary to ascertain if wild yeasts are present, the same method of analysis is employed as already described with regard to brewery yeasts—namely, to take a sample at the close of the fermentation and make a spore culture on a block of plaster of Paris. With many types of distillery yeast the structure of the spores is not at all, or only very slightly, different from that of wild yeast spores ; other tests must therefore be employed—for example, the length of time required for the development of the spores. For this purpose, it is necessary first to ascertain the length of time required for the development of spores of the type of yeast selected for use in practice. The information is obtained by experimenting under absolutely sterile conditions as follows : A few drops of the pure yeast from young flask cultures are placed on several plaster of Paris blocks, and cultures obtained at varying temperatures—for example : 10°–12° C. (50°–54° F.),

15° C. (60° F.), 25° C. (77° F.), and even 30° C. (86° F.). A comparison with the tables for sporulation of Ellipsoideus and Pastorianus yeasts, published in "Micro-organisms and Fermentation," will be a preliminary guide in selecting the temperature at which to compare the sporulation of the selected race of yeast and the wild yeast. A temperature should be selected at which the wild yeasts can form spores earlier than the selected culture yeast. As has been already stated, this analysis is only of subordinate value.

Experience has shown that the most dangerous enemies of this branch of the fermentation industries are the *acetic acid bacteria and different species of Mycoderma.* As regards *pressed yeast, moulds* are dangerous, as are also Mycoderma, putrefactive bacteria, and wild lactic acid bacteria, which tend to give the yeast a flocky consistence. It is not possible to control the extent of the infection by a simple microscopical examination.

The simplest means for controlling the organisms that easily grow on the surface of the wort, etc., is to prepare plate cultures of wort gelatine. This is done by mixing with the wort gelatine when melted a few drops of the sample, thoroughly well stirred in to ensure good distribution, and then pouring it on to a Petri dish. It is also often necessary first to dilute the sample by mixing a few drops of it in sterile water and then thoroughly shaking it, to ensure the cells being well separated. The cells of Mycoderma will develop in the course of a few days dull, greyish, disc-like colonies, whereas acetic acid bacteria develop transparent, slightly opalescent colonies like small specks; and the mould spores develop colonies throwing out many small branches from their edges.

The approximate amount of an infection by *Mycoderma* can be estimated by the following accurate method: On

a solid medium (unhopped wort with an addition of 5 per cent. gelatine and 1½ per cent. agar) one prepares a *superficial culture*, that is, one in which all the cells develop on the surface; by this means the peculiarities of all the colonies are caused to manifest themselves very distinctly, and the large colonies of Mycoderma can be easily distinguished from those of yeast. By taking small samples from different parts of the yeast mass or of the mash, and transferring them into sterilised water, an average sample is obtained after shaking up well; the latter is diluted several times, say in some 10 c.c. flasks, with sterilised water, and the last dilution is poured along the surface of some gelatine-agar that has solidified in a Petri dish. Subsequently the water is poured off and the dish, covered with its lid, is placed in a thermostat (1 or 2 days at 25–27° C.). It can then be easily perceived with the naked eye which colonies consist of yeast and which of Mycoderma.

A weaker infection of *acetic acid bacteria* in the liquid will often not give distinct growths on plate cultures of wort gelatine. Several Freudenreich flasks containing wort and a little alcohol added must then be infected with the wort sufficiently diluted with sterilised water, and an examination made of the film that will form on the surface. The presence of acetic acid bacteria can always be ascertained by their characteristic growth as well as by the smell of the liquid.

In addition to the culture yeast, cultures of *lactic acid bacteria* are also employed in this branch of the fermentation industry. As undesirable species of bacteria are always likely to establish themselves during this kind of alcoholic fermentation, their suppression is always attempted by encouraging the growth of lactic acid bacteria.*

* We leave completely out of the question the importance or not of lactic acid bacteria in other directions.

As it is important to check foreign germs during the early stages of their growth, the development of lactic acid bacteria must be commenced at a very early stage of the fermentation, namely, immediately after saccharification. The growth and activity of the lactic acid bacteria are checked by the lactic acid, and cease at last altogether, the bacteria being quite crippled. In distilleries and yeast manufactories the lactic acid fermentation is confined to the acid mash after saccharification, and concluded with this stage of the process in order that growing bacteria may not be introduced into the principal mash. The latter must not be allowed to develop considerable quantities of acid (Max. : 0·2°).

Experience therefore lays down the following principles for our guidance in carrying out an analysis at the different stages.

An analysis of the acid mash at the first stage, when acid is being formed, should reveal the presence of a healthy, vigorous development of lactic acid bacteria, and only a very slight development (if any at all) of other organisms. In the fermented acid mash, and in the principal mash started with the yeast developed in the acid mash, the lactic acid bacteria will, with the aid of a microscope, be found to be still present, but so crippled that, on making cultures on sweet wort gelatine, there will only be a very weak growth, if any at all.

When making an examination for acid bacteria, plate cultures are made, using either the usual wort or dextrose solution and gelatine ; or a small quantity of precipitated calcium carbonate is mixed in with the nutritive gelatine. In the latter case the gelatine has a milky appearance. The colonies developed by the acid bacteria have the power of making the gelatine transparent in their immediate neighbourhood, as has been described, and they appear

to be surrounded by a transparent ring, because the acid dissolves the particles of lime ; thus, partly with the aid of a microscope, partly with the naked eye, it is possible to distinguish whether the colonies are developed by acetic acid or lactic acid bacteria. The former are also sometimes able to produce a transparent zone.

The presence of *wild lactic acid bacteria* is demonstrated by making from the material a plate culture on sweet wort with an addition of agar-gelatine and chalk and leaving it to stand at 25° C. At this temperature the wild lactic bacteria will develop, whereas the culture lactic ferments will be stinted in growth. From the acetic acid bacteria, which may also develop under these conditions, they differ by the appearance of their colonies. Whilst the former group forms thin, large, irregular, faintly bluish colonies on the surface, those of the wild lactic bacteria appear as sharply outlined white dots or speckles.

In distilleries and yeast factories, where the yeast is skimmed off the surface of the fermenting liquid in the tuns, the following analyses are made :

1. **Examination of the Acid Mash during Fermentation.**—The necessary biological analysis in this case is to show that a predominating development of lactic acid bacteria has taken place.

2. **Examination of the Acid Mash after Alcoholic Fermentation** (the yeast all ready for pitching).—A growth of lactic acid bacteria should be present, but be unable to develop colonies on gelatine plates ; the yeast should have grown to a definite extent and should be ripe in a certain measure, proved by the cells having a uniform appearance, particularly as regards their size—cells producing small buds should be the exception—and their contents ; the appearance of the contents of the cell depends to a certain extent on how long fermentation has been

allowed to proceed. What is best in this respect cannot be decided by theory, but only, as already stated, by actual careful experiment. Besides the composition of the mash, and the manner in which fermentation is conducted, the type of yeast employed also plays an important part without doubt. Types of yeast are found in distilleries, as in breweries, which suffer if their action is arrested at too early a stage of development. If that is done constantly, they will be gradually reduced to a feeble state.

3. **The Principal Mash or Wort before Fermentation commences** contains only weakened micro-organisms, and should consequently *not show any development* of the same.

4. **The Principal Mash during and after Fermentation.**—Lactic acid bacteria are to be found with the help of the microscope, but they are not capable of development; they are generally so crippled that they cannot be grown on gelatine plates. The yeast should during the time that fermentation lasts show a uniform development of the contents of the cell. A sample may be taken for spore culture. Samples taken from the surface of the fermenting liquid may be examined to ascertain if Mycoderma, acetic acid bacteria, and moulds are present.

5. **The Skimmed Yeast, and Washed and Pressed Yeast.**—Samples should be compared, as usual, to ascertain if the yeast cells maintain a normal and uniform appearance, and if the water has introduced any infection—that is to say, if in washing putrefactive bacteria have been added and developed. The samples must also be examined with regard to Mycoderma and wild lactic bacteria.

6. **The Yeast used for Pitching.**—This should be examined to ascertain if any undesired development of bacteria, or film-forming organisms, has taken place.

In *air-yeast factories* the following samples are collected:

(1) A sample from the lactic mash before warming.

(2) A sample from the lactic wort before the introduction of the yeast.

(3) The fermented wort containing the yeast that has been grown.

(4) The (washed and) pressed yeast.

The most important points in the analysis have been given already.

The process employed in air-yeast factories affords a good example of how a type of yeast can in practice develop a new form. Under the powerful influence of the air that is roused in, a large or small proportion of the cells, sometimes all the cells, will assume an oblong shape.

It is often not possible to distinguish by a simple microscopical examination the cells of Mycoderma from the yeast cells grown in air-yeast factories. They may show the same clear cell contents; or all cells (Saccharomyces and Mycoderma) may have large vacuoles, and simply a layer of plasma near the walls of the cell. It is therefore always important to make plate cultures as described above, at the same time obtaining information about the bacteria present.

Also, the cells of many types of Oidium, Chalara, and Dematium, etc., are difficult to discover in a field under the microscope, and their numbers can only be estimated by taking a good average sample and making a plate culture with it.

In order to be enabled to judge of the quality of a yeast, it is of some consequence to estimate roughly the amount of *glycogen* contained in the cells. For this purpose the preparation is treated with a weak solution of iodine-iodate of potassium (say 0·4 grm. of iodine per 100 c.c.):

if glycogen is present, the cells assume a red-brown tinge; if not, they are coloured yellow.

Where, as is frequently the case, particular modifications are introduced in the conduct of distilling operations and in yeast factories, this circumstance must be taken into consideration in selecting samples for analysis.

III. ANALYSIS OF WINE YEAST

Even if the acidity and the contents of sugar can be regulated, the composition of the must will show differences from year to year and the wine manufacturer is consequently working with a material subject to many more changes than the brewer's wort.

It is therefore much more difficult to find a yeast race which works satisfactorily and the selection of such a yeast demands great labour and skill. In selecting yeast for the production of wine one must give consideration to the amount of alcohol it shall produce, to its ability to agglutinate, to produce "bouquet," and finally to the fact that it often must work in a must treated with sulphurous acid.

The yeast should be able to endure the sulphurous acid and help to change the free acid into combined acid.

As has been noticed, the spontaneous fermentations often are carried through by means of different yeasts, and fermentations with pure cultures have successfully been accomplished with two different races: The one having started the fermentation, and the other having finished it.

In examining the fermented must from grapes, the first point on which information is desired is whether a development of bacteria is going on in it.

Furthermore, information is desired as to whether at any particular advanced stage of the fermentation the

yeast cells enter into a state of rest, the liquid thus becoming rather still, or whether the yeast is *still growing*.

When a selected type of *pure yeast* is added to wine must, it should be ascertained whether the type of culture yeast is powerful enough to gain and keep the mastery over types of yeast that have crept into the must.

When the different types of micro-organisms are studied individually, to be used for purposes of experiment, the culture must be submitted to a fractional examination, and absolutely pure cultures must be used for the purpose.

The question of infection, owing to the presence of *bacteria*, can only be determined by a thorough examination with the microscope. There can never be any certainty that the bacteria developed in the wine will grow in plate cultures or in nutrient liquids; with the help of the microscope a real growth of bacteria can be proved to be taking place, whilst plate cultures prepared at the same time give either a negative result or results that are inconclusive and of little value. But many of the bacteria found in wine are very small—the cocci, for example—and it is therefore necessary, when using the microscope, to use a good immersion objective. It stands to reason that, with attentive watching, it can be ascertained if a growth is taking place at the moment or not.

The other question of a practical nature—whether *the yeast in the liquid* is always *growing*, and if *vigorous* cells are to be found, or if development has ceased—can likewise usually best be determined with the aid of the microscope, and not by making plate cultures. Naturally, even very exhausted cells, that are, to a certain extent, of no use in wine, can be resuscitated in fluids that suit them. But no conclusions of service in practice can be drawn from this. The growth must be subjected to a direct examination.

Only when no budding cells, no cells with clear plasma, or no uniformly vacuoled cells are found, but only cells that are much granulated, and whose contents are very refractive, containing also irregular large and small vacuoles, then only can it be said that the yeast has entered into a resting state. The question should not be answered at once with a single examination; but the sample must be allowed to remain for some days at a suitable temperature, and then be again examined.

If the wine contains cells of *Mycoderma* capable of germination, they will soon make themselves apparent. If the sample bottles are closed with several sheets of filter paper, and allowed to stand for a very few days only at room temperature, on examination of a drop taken from the surface of the wine, budding cells of Mycoderma will be found.

If *a selected race of yeast* is employed, the difficulty is to add just sufficient yeast to suppress the germs contained in the must. On the other hand, there must not be so much yeast added that the primary fermentation will be too vigorous, entailing the risk of the yeast becoming too quickly exhausted, and consequently, at the later stages of the fermentation, being so weak that it cannot hinder a secondary development of other yeasts or bacteria. This difficulty is increased by the fact that the quantity of natural yeast appearing on the grapes varies in different years. One solution of the question is to commence with a small quantity of yeast, and to take notes of the rate of growth and the course of the fermentation. After a short time conclusions may be formed, and taken as a guide for future work.

Also the quality of the must has to be borne in mind when deciding on the quantity of yeast that should be used.

When the vigorous fermentation is over, an examination must be made to ascertain *if the selected type of yeast still has the upper hand.* A microscopical examination will not afford the information. If the yeast examined under the microscope appears very uniform, it is probably the selected culture yeast, because the general appearance of the yeast in spontaneous fermentation is frequently very irregular. But in all cases a closer examination must be made on settled lines. We will content ourselves here with mentioning some of them.

Spore cultures may be made. If the selected race of yeast possesses characteristics that are easily recognisable, for example, in the structure of the spores, and is able under ordinary conditions to build up spores easily, a sample of the yeast taken straight out of the wine during the development of the cells and placed on a gypsum block will afford useful information.

If the selected race of yeast develops spores relatively late or early at a given temperature, it is also possible to determine approximately the extent to which that race of yeast is present in the sample.

If the selected race of yeast has a characteristic appearance when grown on gelatine, and the vegetation contains a sufficient number of cells capable of developing colonies on the gelatine, plate cultures should be also utilised.

Better results are usually obtained when a large number of cells is separated out, and the pure cultures made from them examined and compared with the preserved sample of the pure yeast species kept in reserve. A portion of the sample diluted is therefore used to infect a large number of flasks, and only those flasks used which contain a single yeast colony at the bottom, after being allowed to stand quiet a few days. The appearance of these pure cultures under the microscope often yields

useful information ; also the taste and smell of the liquid, the physical nature of the sedimentary yeast, the length of time the liquid takes to clarify, etc.

I must, however, lay stress again on the fact that in not a few cases it is very difficult to obtain a thoroughly conclusive result by analysis. The examination of the fermented liquid itself must then be the means of determining if the desired result has been obtained.

IV. WATER ANALYSIS

A micro-biological analysis of the water used in the fermentation industries is not yet made use of so generally as it should be. It has been proved that information of the greatest value in practice can be thus obtained.

This analysis is based upon the fact that all the micro-organisms that are able to interfere with the fermentations are actually able to live for some time in water. This has been proved by inoculating the different liquids used in fermentation industries with samples of water. Such germs are therefore apt to slip in with the water and grow freely if circumstances are favourable.

On the other hand, very many micro-organisms that can live in water do not flourish in the liquids used in the industries of fermentation. The danger from germ infection through water does not therefore stand in any direct relation to the number of living germs which it contains.

The information obtained from such an analysis is of limited effect, because the germs found in a water, and the extent to which infection is present, vary from time to time, just as the chemical composition of a water is found to vary.

As a rule, a really disastrous development of the germs living in water takes place when liquids are left quite still :

for example, if any waste liquid, fermented or unfermented, is not quickly removed from the floors, the walls, or the plant itself; in rinsings which may contain wort or beer; in the water tanks, if these are soiled with malt dust, etc.; in old tuns, if they are allowed to stand empty for some time, and the water soaks into the cracks and holes, where some of the residue of the wort is certain to be; or on coolers that have not been properly cleaned.

When samples are sent to the laboratory, and can only be examined some days after being taken, the growth of the micro-organisms present will have spread. The growth, however, is probably restricted to those germs that flourish in water ("water bacteria" so called, in contradistinction to the germs that flourish in wort).

In many places, special water-tanks are used; it is then very important that the water contained in them should not be contaminated. As a general rule, samples of the water must be taken for examination before as well as after the water has been put in.

If it is thought that the pipes are infected, it is advisable for purposes of comparison to obtain samples at the same time from the spring, the well, or the stream whence the water supply is obtained.

Absolutely sterile, clear glass bottles should be used for collecting the samples; flasks that have been sterilised by heating at a temperature of 150° C. (302° F.) for two hours should be sent out (packed in white paper) from the laboratory for the purpose.

The samples of water should be taken from the *tanks* at a time when the water is being roused. When the water is still, some of the germs fall to the bottom; a sample that is taken from the upper layers of the water cannot, if it is not roused, throw any light on the real state of affairs. Whoever takes the sample must

thoroughly wash his hands just before doing so, dry them, and then rub into the skin a mixture of alcohol and water. Immediately before plunging the sample bottle into the water, and no sooner, the sterilised bottle is taken out of the paper; then it is sunk down as deep as possible under the surface of the water before opening, the stopper loosened, and the bottle allowed to fill. When full, close it again tightly whilst still deep under the surface, take it out and bind down the glass stopper, and *fix a label* to the bottle; and lastly, wrap it up again in the sterilised paper from which it has been removed. If a sample is taken from a well, it should be collected in the same manner, so far as circumstances permit.

When a sample of water is to be taken from a tap on a *water-pipe*, the possibility of germs growing and flourishing on the inside of the tap itself must not be overlooked. But the problem is to determine the number of organisms present in the total daily amount of water used. It is therefore necessary to let the water run for some time before taking a sample, in order to be sure of obtaining an average sample. The sample is taken when the water is running at full bore, the bottle, with the stopper still in, being thrust into the stream, then opened, and shut again before it is taken out of the stream of water. Samples are taken from a bore tube (Artesian well) in the same manner.* When the sample has been taken, the bottle is packed in a mixture of crushed ice and sawdust and sent to the laboratory.

The principle underlying this analysis is, that in the laboratory a few drops of water are used to infect liquids of the same nature as the liquid used in practice, but with this

* The different special kinds of flasks that have been proposed for collecting samples of water are only serviceable when specially trained experts can take them.

difference—that the liquids used in the laboratory are sterilised. The brewery wort from the filter bags is usually employed for this purpose in analysing water in the brewery ; it is then sterilised in Freudenreich flasks. Lager beer, sterilised by steam or in boiling water, is also used: the resulting loss of alcohol is made up again. In the case of distilleries and air-yeast factories sweet wort is made use of.

The different yeast types, moulds, and bacteria most able to develop during alcoholic fermentation can be grown in these liquids.

If *exceptional* questions require solution—for example, whether the water in a yeast factory or distillery contains *acetic acid bacteria*—liquids are usually employed that have been found to be particularly favourable for the development of bacteria of this type ; exhausted germs are thus strengthened and their development assisted. A top-fermentation beer that contains a high extract is usually employed for this purpose ; it is only slightly fermented and lightly hopped, and contains about 2 per cent. alcohol.

If it is desired to ascertain if the sample of water contains a large proportion of yeast types that are capable of rapidly *developing films* on *fermented wort* (*e.g.*, *Mycoderma*), or are particularly prevalent in air-yeast factories and quickly and vigorously develop there, it is useful to make use of wort gelatine plate cultures as already described. After the lapse of a few days any germs that are present will have developed small, grey, film-like colonies, and by counting the number of colonies the number of Mycoderma germs in each drop of water can be estimated. Where *acetic acid bacteria* are concerned, plate cultures (wort or dextrose gelatine with an addition of precipitated carbonate of lime) can also be employed. In the case of

moulds—when, for example, it is desirable to ascertain if the water is infected with any germs from the malt-house—ordinary wort gelatine plate cultures are used.

For yeasts and some other organisms the gelatine plates are of no use, because the exhausted cells either cannot grow at all on them, or only show slight growth, no colonies being formed that can be identified as yeast colonies. Only nutritive *liquids* can be used. It is known that the wild yeasts are the most dangerous enemies to all fermentation industries, because they, as a rule, require the same conditions for supporting life as the culture yeasts, and therefore develop when the culture yeasts are growing; consequently, the identification of such organisms is of the greatest importance.

Liquids (wort, beer, neutralised yeast-water, etc.) must also, as a rule, be employed for the identification of Sarcina types that are found in breweries.

After a preliminary microscopical examination of the sample of water, to obtain some idea about the infection from moulds, yeast cells, bacteria, or large quantities of decaying organic matter in the water itself, a sample of the water is distributed by drops over several flasks, both as it is, and diluted with sterilised water in varying strengths.

The object is to introduce only *a few germs* into each flask, in order to ensure the growth of the predominating species taking place in a large number of flasks. The types of micro-organisms present in but a slight quantity would commonly not be developed under these conditions.

The flasks containing nutrient liquids (for breweries, hopped wort, beer; for distilleries and air-yeast factories, sweet wort, beer) are sterilised externally with a flame, and placed in a glass case previously washed inside and out with a solution of sublimate (1 : 1000) or with 50 per cent.

alcohol, and having a door that slides up and down (Fig. 10). The bottle containing the water is well shaken and, by means of a sterilised pipette, partially closed at the

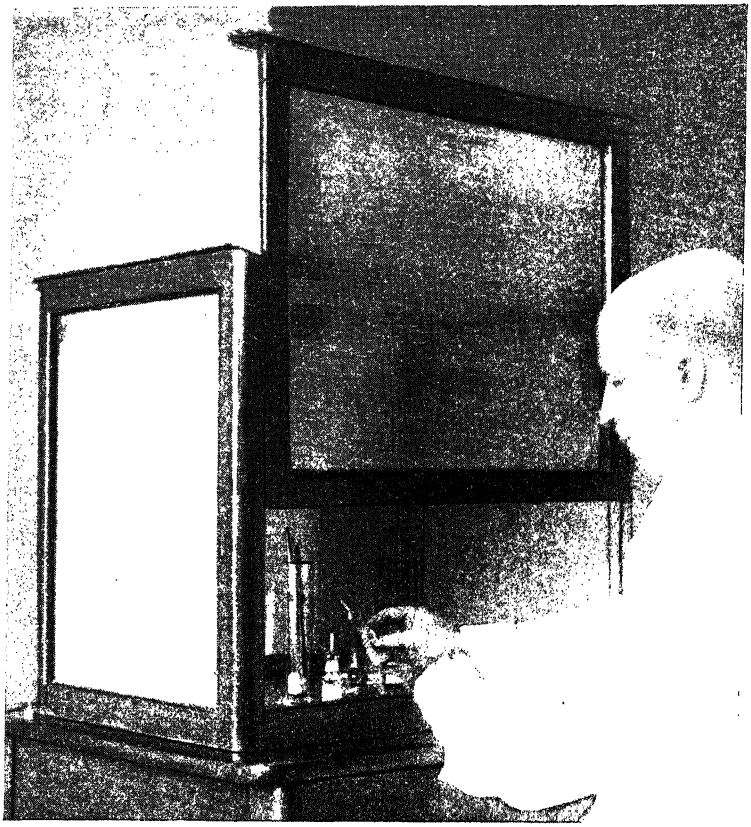


Fig. 10.—Sterilised cupboard, in which work is carried on free from risk of infection from the atmosphere.

upper end with sterilised cotton-wool, only a *very few* drops of the water are taken out after each shaking of the flask and rapidly transferred to the culture flasks, a drop in each flask. The culture flasks are then put into

a thermostat at 25° C. (77° F.), or kept at room temperature; or the flasks can be infected in duplicate, and kept under observation—one series at a constant temperature, and the other under varying conditions of temperature.

The research generally extends over a period of *ten* to *fourteen* days.

Organisms found in water develop in varying degrees in the particular liquids produced in breweries, distilleries, etc. Also, during fermentation, they develop with varying power of resistance in the presence of the culture yeast.

A guide in ascertaining how vigorously the different organisms can develop in the liquid concerned is the length of time that elapses before a growth is formed *that is clearly visible to the naked eye.*

The flasks kept at 25° C. (77° F.), or at room temperature, should be looked over every day, and particular attention should be paid to the clearness and colour of the liquid, to the surface-vegetation, and to the bottom-growth.

From time to time the number of flasks that show a change in the condition of their contents is noted. These flasks should be at once examined microscopically, also biologically, as described in the section devoted to yeast analysis (Part I, section i).

The effect the organisms exercise on the condition of the liquid in the brewery, distillery, etc., when surrounding circumstances are favourable to their growth, stands in direct relation to the vigour with which they are able to develop in the wort, etc., in the small flasks.

In analysing water for fermentation industries, it is often noted that a large number of the wort flasks is infected with a preponderating growth of putrefactive bacteria—at once recognisable by the smell. It is a fact established by experiment that these organisms are not

able to assert themselves during *alcoholic fermentation*, therefore from the fermentation point of view they need not be taken into account when deciding if the water is fit for use. These organisms may be classed as "water bacteria." But still, it must be borne in mind with regard to them that their vigorous development in water betokens that it contains organic matter in a state of decomposition, and there is always the danger that, under unfavourable circumstances—*e.g.* infection of well water brought about by surface water penetrating into it—dangerous organisms may also be able to increase in the water.

For washing yeast in a yeast factory, it is clear that a water containing many putrefactive bacteria should not be used.

It is of particular importance to examine the growth appearing in the flasks *during the last days for which they are left quiet*, because it often happens that the multiplication of *wild yeast* and other organisms which may make their influence felt does not take place till then.

As regards the yeast races employed in factories, it is well known that an inquiry as to their power of resistance towards foreign micro-organisms can be carried out by infecting (with a few drops of the water) flasks containing absolutely sterile wort and the pure yeast type employed. The water should be mixed with the fermenting liquid at different stages—*i.e.* when the yeast is introduced, and at different stages during the fermentation, more particularly during after-fermentation. Fermentation must be carried out at the same temperature as in the factory. It is, however, impossible to work in the laboratory under the same conditions as in the brewery or distillery; definite conclusions cannot therefore be always obtained.

An easy method for examining water by finding its destructive power has been introduced by Schlesinger,

and as it also gives good results within a short time (5 days), it will be mentioned here: 8 Freudenreich flasks filled with sterile wort and 8 flasks filled with sterile beer are required. The flasks are inoculated with decreasing amounts of the water to be examined, from 8 drops to 1 drop. The factor of dilution is consequently increasing from 1 to 8. The time factors, commencing with 2·5 the first day, decrease by 0·5 each day, being 2·0 the second day, 1·5 the third day, 1·0 the fourth day, and 0·5 the fifth day.

The maximal destructive power of water is found to be 90 if all the flasks show development of organisms after one day.

After the flasks have been examined daily for 5 days, the result (wort) is calculated as follows :—

Dilution factor × Time factor = Destructive power.

Flask No. 1 cloudy after 2 days, 1 × 2·0 = 2·0
„ „ 2 „ „ 3 „ 2 × 1·5 = 3·0
„ „ 4 „ „ 5 „ 4 × 0·5 = 2·0
Total destructive power = 7·0

As the organisms develop more slowly in beer, the calculated destructive power is multiplied by the factor 5/3. Judged by its destructive power, a sample of water having such a power from 1–20 is considered as very good; 20–40 is good; 40–60 fairly good; 60–80 bad; over 80 very bad.

Will has proposed to complete Schlesinger's examination of water with a fermentation test: To 10 c.c. wort is added 1 c.c. of the water and 1 drop of liquid yeast (pure yeast). The flask is placed in the thermostat and examined after 6 days. By means of this test it is possible to show whether the water contains harmful germs able to survive the fermentation.

V. AIR ANALYSIS

The number of living germs floating in the air may often amount to several thousand per c.c. The true danger-point for infection, where the fermentation industries are concerned, is rarely in any direct proportion to these high figures. This is accounted for, on the one hand, by the fact that usually only a very small proportion of the micro-organisms present in the air are either able to grow or capable of exerting any influence in the liquids undergoing fermentation; and, on the other hand, because the dangerous organisms are only able to exert any influence when undisturbed, and the occasion is favourable for them to increase in numerous generations, and at the same time to become accustomed to the particular circumstances in which they find themselves.

Such cases occur only, as a rule, when the *thorough* cleaning of the plant is neglected or if an infected yeast is not removed at once without delay.

The germs which have become rooted will then grow to such an extent that they will, under favourable circumstances, be carried about in the air, and an analysis of the air will reveal their presence. They are now no longer air-germs in the ordinary sense of the word.

An expert examination of the state of affairs prevailing in practice will often have exposed the true nesting-place of the objectionable germs long before it is found out by an air analysis.

A comparative analysis of the number of germs growing in brewery wort at the moment when it leaves the coolers, and twenty-four hours later, after the yeast has been growing in the tun, affords a striking example of the fact that even such micro-organisms as can grow in the wort are only able to assert themselves under certain

circumstances. It has been possible, with the help of fractional analysis, to determine the presence of many hundred germs per c.c.—living germs, capable of growing in wort—in the wort on the cooler. Twenty-four hours later, the same wort, in the fermenting tun, showed very slight traces of any growth of these foreign organisms. By far the greater majority had been unable to grow, and thus be recognised in the analysis.

Definite conclusions are therefore only based upon an examination of the fermenting liquid itself, and the surface of all objects in the different rooms.

The analysis of the air may have a very particular object—namely, to ascertain if foreign germs are introduced into the fermenting liquid from any known suspected sources of infection in proximity to the tun room.

Also, in this case, we proceed in a practical manner by making an examination of the suspected sources of infection. For example, if one wishes to know whether the organisms that rapidly make their appearance when the grains are lying exposed to the air, and are often naturally carried up into the air by dry grain particles, are dangerous for the fermentations or not, an answer is best obtained if the wort is infected with some of the grains, and comparative fermentation experiments carefully carried out—every endeavour being made to approximate to the conditions prevailing in practice.

If it is desired to make an experiment in order to ascertain which of the organisms that are in the air, and are deposited *on the coolers*, are capable of influencing the fermentation, the best plan is to fill cylindrical-shaped glasses with some litres of quite pure wort, put them by the coolers, and leave them open whilst the wort is exposed to the air. Add a suitable proportion of absolutely pure yeast, and carry on the fermentation in these cylinders

under conditions identical with those regulating the fermentation in the big tuns. An analysis is made at the end of the primary fermentation and again when the beer drawn off from its sediment has passed through after-fermentation. In many cases we learn from such an examination especially whether the coolers and their immediate surroundings are kept clean or not.

In order that information gained by researches of this kind may be of practical use, a clear understanding of the work in hand must be arrived at between the practical man (brewer, distiller, etc.) and the analytical chemist. The latter must be familiar with the methods of working adopted in practice, and the brewer and distiller must understand the main principles underlying the methods employed in the analytical work.

If the analysis of the samples of air can be carried out on the spot where the samples are taken, no other apparatus is required in practice but the flasks in which the growth of the germs, found in the air, can proceed. A large number of flasks is placed in the room or locality concerned (the ordinary Freudenreich flasks are very suitable), and they are filled either with sterile wort, distillery mash, or must, as the case may be, varying with the industry concerned. Some of the flasks are only open for one or two minutes, and the remainder are kept open for a quarter of an hour or half an hour. Of course, the man who places the flasks in position, opens them, and later closes them must have on clean clothes and have washed his hands clean. The flasks are then allowed to stand at room temperature, or at 25° C. (77° F.). The examination of the cultures obtained is generally closed after two weeks' observation. If a number of the flasks contain a mixture of organisms, it may be important to separate the types.

If the object is to find out whether particularly strong development of moulds, Mycoderma, and acetic acid bacteria is present, ordinary wort gelatine plate cultures can be carried out. The liquefied wort gelatine having been poured out into Petri dishes, the wort gelatine is allowed to set into the closed dish, and the dishes are then exposed on the cooler.

The most important question—whether large numbers of *wild* yeast cells are floating about in the air—can only be answered by resorting to the use of liquids, as many of these cells, when they settle out of the air on the gelatine plates, are not so vigorous that they can develop colonies, and therefore they escape observation. An analysis with the aid of liquid media is, therefore, always the most important, as with the aid of liquid media alone is it possible to discover the most dangerous germs. The reason why *yeast* infection is the most dangerous is that these organisms, as remarked already, flourish well under the same conditions as the culture yeasts, whereas the majority of other organisms are suppressed under the conditions of fermentation that usually prevail.

When it is not possible to carry out an analysis of the air on the spot, the germs must be *collected and despatched* to the laboratory. The following is the most sure method of carrying this out :

Several bottles, containing about 25–50 c.c., are sterilised at the laboratory. They are closed with a conical-shaped india-rubber cork, which only enters the bottle to the extent of one-third of its depth. Some of these bottles contain sterilised water ; the remainder, the liquid that is to be fermented, wort, distillery mash, or whatever it may be. The bottles are placed at different levels in the place where it is desired to make an examination of the air ; the corks are withdrawn with clean hands,

taking care not to touch the rim of the bottles, and are laid down close to the bottles with their inner surface turned up, where they should remain undisturbed. After an interval of between a quarter of or half an hour, the corks are put into the bottles again and tightly fastened down. The bottles are then packed up in clean paper and sent back to the laboratory.

During transit, the germs will increase particularly in the bottles filled with a nutritive medium; some of the predominating types present will therefore be the more easily identified.

On reaching the laboratory, the contents of the bottles are transferred, directly or after being first diluted with sterile water, to flasks containing the nutritive liquid employed in practice, a drop in each flask. A larger quantity of the water is put into some flasks filled with the requisite nutritive liquid; here the different types will be mixed up together and struggling for survival.

The flasks are allowed to stand at a temperature of 25° C. (77° F.), or at room temperature, and submitted, after ten to fourteen days, to the same methods of examination as employed in yeast analysis (Part I, section i).

If it is desired to find the number of germs present in the air in different places in the factory, Koch's plate method can be used, exposing Petri dishes with wort gelatine for a certain time and thus calculating the number of germs falling during a certain time (20 minutes, 30 minutes, or 1 hour) on a known surface (a Petri dish having an area of 63 sq. cm.). In this way it is possible to state how many germs are falling on the surface cooler during the cooling of the wort, or in the fermenting tuns during the fermentation of the wort. Similar results can be obtained by using an aspirator and passing a certain

quantity of air through sterile water or wort, which retains the germs.

VI. NUTRITIVE LIQUIDS AND GELATINE

Only a very small number of nutritive liquids is used for the cultivation of alcohol yeasts and fermentation bacteria in a laboratory where the work chiefly follows practical lines. As the characteristics of different yeast types are relative, and to a certain extent dependent upon the nutritive medium in which they are grown, it follows that attention must be paid to the point—from which branch of the fermentation industry the particular yeast type under examination is obtained.

The most valuable nutritive liquid is certainly *hopped brewery wort.* The majority of yeast types grow freely in this medium. The best plan is to use wort from the coolers that has been filtered (wort from the filter bags), as it only throws down a slight deposit during boiling in the flasks.

The wort is first boiled in large copper cans, Carlsberg vessels, and placed on one side to clarify before it is used in the small flasks.

The large copper cans (*Carlsberg vessels*), Fig. 13, are like the Pasteur flasks, but they are fitted with an outlet pipe at the bottom and a cotton-wool filter fits over the bent tube. These large copper cans are used for growing large quantities of a particular culture, and it is best to sterilise them over a naked flame, both upper tubes being allowed to remain open for an hour, boiling being then continued for another quarter of an hour with the india-rubber connection on the short upper outlet pipe closed. In order to ensure complete sterilisation of the lower india-rubber connection, a little wort is allowed to run out through it whilst boiling is going on, and then the glass

stopper, sterilised in a flame, is again pushed firmly in. Before the cans are removed from off the burners, the cotton-wool filter must be fixed very firmly on to the long bent tube. The boiling is repeated after an interval of 24 hours, but this time only for 30 minutes, the steam escaping through the long bent tube after the cotton filter has been removed. After this final sterilisation, the cotton filter is replaced and the vessel put to one side for a fortnight in order that the sediment may settle to the bottom and the wort absorb oxygen.

The small bottles used are :

(1) Pasteur flasks (1/2–1/4–1/8 litre).

(2) Freudenreich flasks, with or without side-tube, 10–15 c.c.

(3) Small Erlenmeyer flasks, closed with a stopper of ordinary cotton.

The flasks are filled with wort by connecting the side-tube with the rubber tubing of a Carlsberg vessel, or by filling them directly.

The wort is sterilised in steam three times for half an hour, with an interval of 24 hours. The flasks should be kept for some time before being used, partly in order to make sure that they are sterile, and partly in order that a slow oxidation may take place from the surface of the liquid.

If the sterilised flasks are allowed to stand in a closed cupboard and not exposed to any sudden change of temperature, there is no risk of living germs being drawn into the liquid.

When *distillery mash* or *wort* from the *yeast factory* is to be used, neither can be used as it is, as in the case just described, but they must first be filtered. An ordinary sterilisation is attended by the difficulty that these liquids

undergo marked changes, because a large quantity of nitrogenous matter is precipitated out of solution during boiling.

Wine yeasts can in many cases be grown in wort without suffering any deterioration. A little tartaric acid may be added. When *wine must* is required for use and ordinary wine must is not at hand, concentrated (*in vacuo*) must can be purchased from the trade, and then be used diluted with distilled water. A suitable nutritive liquid to grow wine yeasts in can be obtained by using a decoction of raisins, to which a small quantity of tartaric acid or citric acid together with tannic acid has been added. Malt extract or a solution of cane sugar, to which citric acid and ammonium phosphate have been added, can also be used.

Yeast water may be used for testing the yeast in a pure culture machine to ascertain if bacteria are present. Brewer's yeast, preferably bottom yeast, is washed with a 1 per cent. solution of soda, rinsed with water and pressed. 2 kg. of the pressed yeast are then boiled with 20 litres of water for about half an hour. The liquid is left till the following day, when the clear part is withdrawn and distributed in 2 litre flasks. The flasks are sterilised in a current of steam three times with an interval of 24 hours. When about to be used, the *p*H value of the solution is adjusted to 8·2–8·4 by adding concentrated ammonia. After being filtered the solution is distributed in Freudenreich flasks and sterilised as above. When inoculated with yeast a few drops of alcohol and autolyzed yeast are added to each flask. Autolyzed yeast is prepared by breaking 2 kg. of pressed yeast, prepared in the same way as for yeast water, into pieces and mixing it with 10 grms. of salt (NaCl). The yeast is left to stand at 50° C. for 50 to 72 hours. At the beginning foam may appear.

2 kg. yeast, will yield about 400 c.c. extract. The filtrate is neutralised ; it contains 2 per cent. nitrogen (N).

A solution of *dextrose and yeast water* is employed to obtain a stronger growth of the yeasts that do not ferment maltose, as well as for the purpose of resuscitating yeast types that are likely to lose their power of producing spores owing to their constant repeated culture in hopped wort. Ten to fifteen per cent. dextrose is added to the non-neutralised yeast water.

A 10 per cent. solution of *saccharose*, usually clear ultramarine-free crystallised sugar and distilled water, is used for preserving pure yeast cultures. After boiling for a short time the solution is filtered off quite bright and sterilised in the flasks.

Hopped wort, distillery mash, must, dextrose, yeast water, and dextrose and yeast water together, are all used with nutritive *gelatine*. Ten per cent. by weight of gelatine mixed in a little lukewarm water is added to the wort or liquid required. The mixture is warmed, boiled with white of egg, allowed to clear, and filtered through flannel ; the nutritive gelatine is then sterilised in the flasks by being submitted to boiling in a steam bath three times for 20 minutes with an interval of 24 hours. It must be remembered that either too long boiling or repeated boiling may prevent the mixture from gelatinising properly.

Agar-agar is sometimes used instead of gelatine. It is cut into small strips, and allowed to soak in a 1–2 per cent. solution of ordinary salt and water for twenty-four hours before it is required for use. The solution of salt is then poured away, and the agar-agar washed with cold water ; it is then dissolved in the nutritive liquid selected for the purpose, being added to it in the proportion of 1–1½ per cent., and then sterilised in the same way as gelatine

substrates. A mixture of $1\frac{1}{2}$ per cent. of agar and 5 per cent. of gelatine is often used.

The advantage of operating with solid media is that, both when sowing a culture and when taking out a sample, the opening or mouth of the flask can be turned down, and thus any direct infection from germs that are in the air be prevented.

II

THE PURE CULTURE OF ALCOHOL-YEASTS

II

THE PURE CULTURE OF ALCOHOL-YEASTS

GENERAL REMARKS. COLLECTION OF SAMPLES

THE pure culture is raised from a single cell. Such a culture is thus free from all infection, such as bacteria and wild yeasts, and it also possesses the condition that makes it possible for the yeast to maintain its character unchanged because the cells are all of one and the same species; so far as we know at present, it is absolutely impossible, by mixing several species of yeast together, to secure a reliable culture capable of keeping unaltered for a length of time. After such a mixture has been used in some fermentations, it is impossible to know in what proportions the different species exist together; and careful research has furthermore shown that one or another of the component types will sooner or later gain the mastery and become the predominating species. Such a mixture has therefore again and again to be prepared afresh.

In some isolated cases brewers have expressed the opinion that beer fermented with a single species of yeast does not possess its former brilliancy. Whenever this objection has been raised, the question has been very carefully inquired into, and it has always been found that the fault has nothing to do with the selected species of yeast, but has been traced back to the wort. In such

cases a faulty conversion of the starch has been often noticeable, but it could not be detected in the course of the ordinary brewing operations; it can only be found out by careful microscopic examination of preparations mounted in iodine solution.

At the same time that these small irregular starch particles were found to be present, it was sometimes noticed that it became impossible for the yeast to increase sufficiently during fermentation. Whether or not there lies a causal relation between these two facts is not yet known for certain, but it is probable.

Formerly the complaint was raised on behalf of bottom fermentation, as well as on behalf of top fermentation, that the employment of a single race of yeast did not allow of a satisfactory secondary fermentation being carried through. Practice has shown it to be possible to find, by careful selection, a type of yeast that will, whether the fermentation be "bottom" or "top," when proper attention is given to the fermentations, carry through the secondary frementation also.

However, certain Torula species are sometimes added, especially to top-fermentation beer, for the purpose of imparting a particular flavour to it.

As a rule, the type of yeast selected from the mixed yeast mass to form the starting-point for the pure culture in mass will be, if the samples are taken in the proper manner, the predominating type in the mixed yeast and the type that gives the mixed yeast its particular character. But the single cells of this type are not found to be identical. One would not achieve what *can* be achieved, if one employed a yeast raised from a single cell of the predominating type, selected only by means of botanical and other characteristics. As, on the one hand, it is not an easy matter to define the limits of the species, conjectures

more or less accurate can alone be made ; and, on the other hand, in individual cultures of a group which may be regarded as belonging to one and the same type, differences are to be noticed which can be traced back to peculiarities innate in the cells from which these cultures were originally raised. It is therefore necessary to make a choice between these different *varieties*, and to seek to find out the particular variety that in practice will be able to produce the proper taste, smell, degree of attenuation of the beer or wine, and proper yield of alcohol in the case of distilleries, or the highest yield of yeast in yeast factories, etc.

This selection must in any case be first carried out in the laboratory. The preparation of pure cultures thus demands not only skill in carrying out trials, but also practical insight into the particular branch of fermentation industry concerned. Certainly, experience can alone guide one in selecting the particular type from which the pure culture is to be grown.

The procedure adopted in taking the sample from which the pure culture will be raised is a very important matter.

With regard to *brewery bottom-fermentation yeasts*, we know that at the end of the primary fermentation wild yeasts can develop strongly in the beer, that at this stage the culture yeast will soon cease increasing, and that the culture yeast cells are to a certain extent exhausted. During the first stages of the fermentation the culture yeast is the predominant type, and in a state of lively and vigorous development. And, as it is from this yeast that one wishes to raise the pure culture, either a sample of the fermenting wort can be taken during the first stages and the cells floating in it made use of ; or, what is often done, after the fermentation is finished and the beer has been racked off, a sample can be taken from the *middle* layers of the sedimentary yeast left in the tun. A few drops

of this thick-fluid yeast are collected either on sterilised filter paper or in sterilised bottles sent from the laboratory for that particular purpose.

The samples of *top-fermentation yeast* are taken from the strong yeasty heads thrown up in the tun, or sometimes in cask, as the case may be. When a yeast that works very slowly over a long period is required, the right thing is to wait to take the sample until the quieter fermentation has set in, as it is highly probable that the races of yeast that work quietly are then predominating.

The samples of *distillery yeast* are taken from the yeasty head in the fermenting rounds. In the air-yeast factories pressed yeast is made use of.

Wine yeast samples are taken at different times during the fermentation, bearing in mind that the types that play an important part in the process do not always develop during the earliest stage of the fermentation. Samples are taken from the liquid itself, and also from the sedimentary yeast, using a pipette—a thin pointed glass tube—for the purpose.

I. THE PREPARATION OF A PURE CULTURE

Before making use of the samples sent to the laboratory, an examination should be made to see if the cells are vigorous or exhausted.

As a rule, one type of yeast predominates in brewery yeast. If all that is desired is to obtain a pure culture of this type, and the cells in the sample are vigorous, they can be introduced directly into the moist chamber; but if they appear exhausted after the journey, the culture is sown in a nutritive liquid. The preponderating type will be the first to grow, and the young culture is used directly the first signs of fermentation are visible.

In distilleries and wine factories, on the other hand, several types are often found all growing freely at the same time. It is then necessary to make a pure culture of them all in order to make a comparison between them. In order to carry this out accurately, the yeast sample is used to infect a number of flasks, after first diluting it considerably with water, so that each drop of water may contain only one cell, or at the most only a few cells. Fifty or more flasks are then sown, one drop only of the sample being sown in each flask. The several more or less predominating species have then been separated from one another, and a selection among the flasks is now

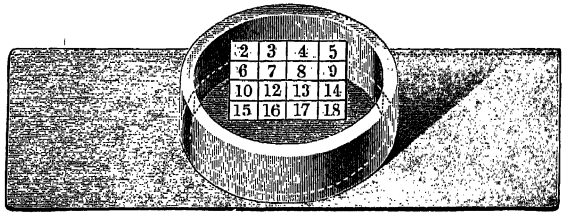


Fig. 11.—Moist Chamber.

proceeded with, some of which are used for the preparation of single cell cultures in a moist chamber.

The *moist chamber* (Fig. 11) is made by fixing a cover slip, ruled out with numbered squares, to a glass ring with wax, and it is then mounted on an ordinary glass slide with vaseline. About fifty colonies can be grown on this cover slip, all well separated from each other.

After the yeast has been sufficiently diluted with wort or water, one or more drops of the sample are passed over to a small flask containing wort gelatine, the wort gelatine having been kept for a considerable length of time in a water bath at 28° C. (82° F.). The flask containing the yeast and gelatine is well shaken for a long while—frothing must, however, be avoided—and well stirred with a

sterilised glass rod; a drop of the gelatine is then placed upon an ordinary glass slide, covered with a cover slip, and the preparation searched over under the microscope, using a low power (100 magnification) and little illumination. If cells are found here and there without any difficulty, the mixture of wort gelatine and cells which has been standing in the water bath during this examination is fit for use. (If there are too many or too few cells, it stands to reason that the sample must be either diluted with more nutritive gelatine or more yeast must be added, as the case may be.) Shake the flask again, stir it vigorously with the rod, and place a drop of the gelatine on the under side of the cover slip of the moist chamber, turned face up, cover over the moist chamber, lying face upwards, with a sterilised glass plate, and leave the gelatine to set. The glass slide is then ringed with vaseline according to the size of the moist chamber, and the ring is pressed tightly down on to the vaseline. The position of a number of isolated single cells is noted down under the microscope. The squares and their numbers are drawn out on a piece of paper, and the corresponding positions of these single cells are noted down on the paper by little dots. Then a drop of sterilised water is placed on the bottom of the chamber. This plan of marking down the position of cells with the aid of figures in the large squares of the cover was introduced by the author in 1883 in order to do away with object markers.

The growth of the marked cells is now kept under observation.—It is always possible when searching for single cells to overlook small budding cells, or bacteria that will be able to grow in the gelatine. When such small cells or bacteria lie close to the marked cells, and possibly develop small colonies, they may eventually be absorbed by the larger yeast colonies. When, however,

the growth of the yeast cells is kept carefully under observation, the small colonies whose growth in the neighbourhood of the large colonies may render the latter useless are quickly discovered.

As a rule, three days' cultivation at room temperature is necessary to produce colonies large enough to be taken out of the chamber and placed in flasks.

The Pasteur flasks previously sterilised on the surface by passing them through a flame are placed in the glass case (Fig. 10), also the moist chamber and a small metal or glass box or tray containing platinum or copper needles that have been heated red hot and allowed to cool, and a pair of sterilised forceps are placed in the case in front of the operator. The glass ring with the cover slip on top is carefully lifted from the glass slide, and a needle held firmly with the forceps is put in from underneath, and one of the marked colonies, whose position is easily located with the help of the numbered squares, taken off on the point of the needle. The needle, with colony attached, is dropped into the flask, and the glass ring is placed back again on the slide.

When a sufficient number of flasks has been sown, each with one colony, they are gently shaken to disperse the yeast cells, and placed on one side at room temperature or 25° C. (77° F.).

When it is necessary to grow large quantities of yeast for use in breweries, distilleries, etc., the colonies can be introduced into $\frac{1}{8}$ litre ($\frac{1}{4}$ pt.) Pasteur flasks; otherwise Freudenreich flasks can be used. The propagation of the yeast in larger flasks is not proceeded with until the cultures have been developed to their *fullest extent* in the Pasteur flasks—that is, until the greater part of the yeast has settled at the bottom. Opportunities for making observations of a practical nature already present

themselves in the course of this first fermentation—the time that elapses before the yeast is deposited, the nature of the foam on the surface, and particularly the nature and colour of the sedimentary yeast, etc. (whether compact or loose, white, yellow, etc.), should all be taken note of.

When work is being carried on with brewery yeasts, it must be borne in mind that not only certain types of wild yeasts, but also certain types of culture yeasts, settle down or rise to the surface with difficulty, and form a very loose-lying sediment in the flask of sterile wort.

There are both top-fermentation and bottom-fermentation yeasts amongst the types suitable for carrying on the fermentations in distilleries, and both kinds of yeast are in use in practice.

Also with wine yeast, differences are noticed in these flasks according to the appearance of the fermentations.

The yeast developed in the first series of flasks should be used to obtain young cultures in a second series; and from this second series samples are taken for microscopical examination and for spore-cultures. The information thus obtained enables one to arrange the pure cultures according to any particular characteristics they may present biologically and botanically.

Some idea is thus obtained as to which types predominate under these conditions.

Valuable information is obtainable by careful examination of the structure of the spores. Distinct differences are observable if the spore-formation is allowed to proceed slowly on the gypsum blocks at low temperature.

After the cultures have been carried through a few fermentations in the Pasteur flasks, comparisons of growth are made by introducing the yeast into cylindrical glasses, containing one or two litres ($1\frac{3}{4}$–$3\frac{1}{2}$ pts.) of sterile wort, and closed with two or three layers of sterilised filter paper.

A little of the yeast in the small flasks is grown on in larger Pasteur flasks for this purpose, the yeast grown in these larger flasks is transferred to the cylindrical glasses, and the yeast that remains over in the small flasks is put on one side for use later on, if necessary, or some of it can be used at once for obtaining fresh fermentations.

The cylindrical glasses are thoroughly cleaned and dried, a little alcohol is then poured into them and set alight to sterilise them, and filter paper is then placed over the open ends. The wort is drawn from a Carlsberg copper vessel (Fig. 13). As these experiments are made to obtain comparisons between the yeasts, it is necessary to supply all the cylinder glasses with wort from the same copper vessel: when the wort from two or more vessels is used, it must then be mixed up in the same proportion in each cylinder glass.

There is something in the fermentation in the Pasteur flasks that is like the features of wine fermentation; such flasks can therefore be used when experimenting with wine yeasts. The fermentations in the cylinder glasses, on the contrary, offer a greater analogy to those carried out in breweries and distilleries; but the glasses must be closed over. These fermentations are carried on at ordinary room temperature, or on a large water bath, and all the different features of the fermentation are carefully noted—*i.e.* when fermentation commences; the appearance of the head; the colour of the head, which can be very different according to the amount of coagulable matter thrown out of solution as the fermentation proceeds; whether the fermentation is a top or bottom one; the appearance of the liquid during fermentation (the colour can vary from bright yellow to dark brown); the length of time that elapses before the fermented liquid is quite clear. The attenuation should be recorded, but

only conclusions of a limited nature can of course be drawn from these figures. Of particular importance when comparing beer yeasts is a thorough comparison on practical lines, of *the taste and smell* of the fermented liquid. Experience has taught us that fairly reliable conclusions can be drawn from these small experiments as to the behaviour of the types on a large scale in practice. Some experience and taste for the work are required to carry out these practical tests properly.

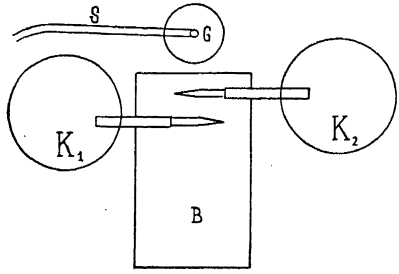


Fig. 12.

The earliest researches of the author with regard to *top-fermentation brewery yeasts* (1884–85) have already shown that, by means of such a series of comparative experiments, it is possible to differentiate between culture yeasts that quickly cease to carry on fermentation and, when no particular measures are taken to assist it, only produce a very feeble after-fermentation, and, on the other hand, other types, that carry on their work over a longer period of time and, under the usual conditions prevailing in practice, are able to carry through an after-fermentation as well as a primary fermentation.

After the choice of a yeast has been made, return again to the flask containing the original pure culture of that

type, transfer it to another flask of the same size, or freshen up the yeast in the same flask with fresh wort, and distribute the young yeast after it is grown, as already explained, into three or five $\frac{1}{2}$ litre ($\frac{3}{4}$ pt.) flasks.*

By permission of Urban & Schwarzenberg, editors of Alb. Klöcker's book, "The Fermentation Organisms," we give below Klöcker's excellent description of manipulating Pasteur flasks :—

"Arrangement of two Pasteur flasks (viewed from above) (see Fig. 12) :

S—Rubber tubing.

G—Bunsen burner.

K_1 and K_2—The flasks.

B—Copper dish.

T—Edge of work table.

"In case a liquid with or without a growth in it is to be transferred from one Pasteur flask into another without being infected by the air, one should proceed as follows : The table on which we are working must be washed with a 60 per cent. alcoholic solution, as well as the Bunsen burner and the rubber tubing. The Bunsen burner (the rubber tubing should be connected to the left) is placed directly in front and between it and the operator a tinned copper dish, cleansed with the flame. The flask from which the liquid is to be poured is placed on the left, the other on the right and both flasks as near the burner as possible. Between both of them is the copper dish. (See Fig. 12.) K_1 is the flask from which the liquid is to be poured into K_2. Both flasks and their supports are now carefully sterilised on the outer surface with the flame ; in case the flasks contain

* A small trace of the young culture is also placed in a flask of saccharose solution and preserved,

organisms this must be done with the greatest care in order not to kill the organisms with the heat. The bent tube of the flask K_1 is now heated up till it begins to glow, commencing at the bulb in the middle of the tube and going downwards to the end. The asbestos plug is then removed. If it is wished to transfer an average sample the flask is shaken up, the lower bend of the tube being held in the flame during the shaking up, so that the air passing in may be sterilised. Care must be taken that the point of the tube is kept out of the flame, otherwise gas would be sucked into the flask, causing an explosion. The gas flame is again put in its place and the glass plug of flask K_2 loosened, without being taken out (if this is done it must be in the flame) so that it remains only with its end fitting loosely in the rubber tube. The Bunsen burner is now adjusted so as to give a luminous flame, because the latter is not productive of so much heat as the non-luminous. The flask K_1 is now taken with the left hand taking hold of the body of the flask, and the rubber of the side-tube is loosened with the right hand. The rubber is then quickly removed in the flame and laid in the copper dish, the opening of the side-tube remaining in the flame while the tube of flask K_2 is squeezed with the right hand so that its glass plug drops into the copper dish. The situation is now as follows :—The left hand is holding K_1 so that the opening of its side-tube is in the rubber tube of K_2; then the side-tube of K_1 is fitted into the rubber of K_2 in the flame. This should all be done so quickly that neither the tube nor the rubber is overheated. The two flasks are now in communication with each other and we leave them in this position without transferring any of the liquid so that the heated tube of K_1 may cool down. This takes place in a short time and the burner, the flame of which is again made non-

luminous, is now held in the left hand and the bent side-tube of K_1 made red hot, after which K_1 is tilted so that the liquid can run from it into K_2, the bent tube of K_1 being heated as long as the liquid is being poured, so that the air passing in may be sterilised. When the required quantity of liquid has been passed over, the Bunsen burner is put back in its place. The glass plug of K_2 is now picked up from the dish with the right hand and sterilised in the flame now made luminous; holding the glass plug between the second and third fingers of the right hand, the rubber of K_2 is held between the first and the second fingers of the same hand and the side-tube of K_1 is disconnected, being placed quickly in the flame; at the same time the glass stopper is inserted in the rubber of K_2 and the rubber of K_1, carrying its glass stopper, is at once lifted out of the copper dish with the right hand and placed on the side-tube of K_1, the opening of which is still in the flame."

The contents of a $\frac{1}{8}$ litre ($\frac{1}{4}$ pt.) flask may be distributed over three to five $\frac{1}{2}$ litre ($\frac{3}{4}$ pt.) flasks, according to the type of yeast being developed. The next step is to use the yeast grown in the Pasteur flasks to infect an equal number of Carlsberg vessels (Fig. 13), each holding about 10 litres (2 gals.). The yeast is introduced through the tube at the top, and the fermented liquid, and the yeast that has been grown, are withdrawn through the outlet pipe at the bottom. A culture for commercial use is usually grown on in three or four, or even five, such vessels, according to the type of yeast.

The Carlsberg vessels are used in the following manner: After making a microscopical examination of the wort in the can, withdrawing for the purpose a small sample through the pipe at the bottom, the side-tube of the Pasteur flask is connected with the india-rubber tubing

of the small pipe on the sloping top of the Carlsberg vessel just in the same way as the glass flasks are connected up, and the yeast is poured in, the bent tube of the glass flask being kept heated very hot whilst doing so. The tightly fitting cotton-wool filter must be removed from the long pipe of the Carlsberg vessel before pouring in the yeast, a loose fitting stopper of sterile cotton-wool being put into the pipe in place of the filter, after the introduction

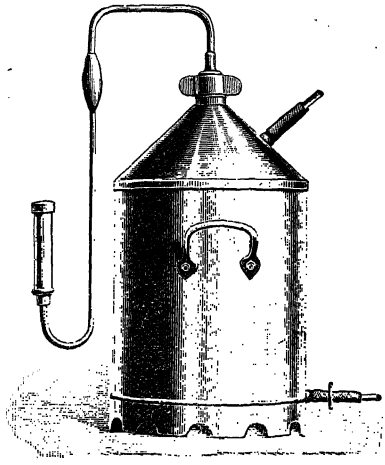


Fig. 13.—Carlsberg vessel.

of the yeast. The vessel is then placed on one side in any convenient place where the temperature is suitable.

After the yeast has settled down in these large vessels, usually after five to seven days, the liquid is withdrawn, the bent pipe being kept very hot whilst doing so. After the tubing has been closed again with the clips, the exposed piece of the tubing in front is dried inside with a spill of filter paper ; and the glass stopper, after being well heated, is pushed in again to dry the tubing and clean it. Sterilised water is then poured into the can through

the side pipe at the top from a Pasteur flask. The yeast is shaken up and a sample drawn out for microscopical examination, and the contents of the three or five copper cans transferred to a travelling can connected up with the lower pipe of the copper can.

The travelling can (Fig. 15) is closed with a cotton-wool filter, if the culture is to be introduced into a pure-culture machine.

Such a pure culture can be preserved for a long time. If there is no use for it directly it arrives, it can be left in the box in which it is packed for the journey, and which must be enclosed in ice.

Such cultures were sent out from the author's laboratory in 1885 for the first time to breweries in tropical countries, and the experiment was completely successful. It is therefore possible to supply breweries, distilleries, etc., in all parts of the world with absolutely pure cultures. It stands to reason that the yeast will be more or less exhausted after a long journey, therefore the first culture in the brewery or distillery must be grown in a very small quantity of nutritive liquid at the favourable temperature of 20°–25° C. (68°–77° F.).

If cultures are to stand a long transport by land, where nothing like the ice-chamber of a steamer is at disposal, they are *dried* in a state of absolute purity.

For laboratory purposes the cultures are sent out on sterilised *cotton-wool* in Hansen flasks (Fig. 9, left). The Hansen flask is attached to the Pasteur flask containing the pure culture, and a few drops of the yeast are poured on to the cotton-wool. On arrival at the laboratory the little flask is connected with a Pasteur flask containing aerated sterilised nutritive liquid, and the yeast on the cotton-wool is rinsed into it.

II. EMPLOYMENT OF PURE CULTURES IN PRACTICE

(*a*) The Development of the Culture in a Small Tun

The introduction of pure cultures in alcoholic fermentations has obtained recognition as an important advance in all branches of the industry. Attention has sometimes been drawn to the fact that a certain time must elapse before the pure culture works in the same way as the ordinary commercial yeast, and this has been stated to be a weak point in the pure-culture system. The pure cultures require a certain time to accustom themselves to the conditions obtaining in fermentation industries; they must be gradually *acclimatised.* But this is the case with every yeast mass, pure or impure, that is introduced in the brewery or distillery; it is not a special characteristic of the pure cultivated yeast.

The pure culture does not always work during the first fermentations, as regards *outward observable conditions*, in full accord with the usual rules laid down for the conduct of the fermentations in the factory where it is introduced. This is often, however, also the case where only an ordinary change of yeast is introduced in breweries and in distilleries, and one is accustomed in the latter case only to regard the fact as something quite natural, which it certainly is. But in the case of the pure culture, there is this further difference that it must be developed in a *small* tun before introducing it into the ordinary large tuns, and it is a well-known fact that fermentation conducted on such a small scale always exhibits different outward phenomena from those observed in the big tuns. When, however, the race of pure yeast has been properly selected and suits the local requirements, the variations noticeable

in the external appearance of the first few fermentations will have no real importance, and by carefully considered management the proper working of the yeast in the large tuns can be secured.

It is self-evident that the new type of yeast must be proved to be satisfactory for the establishment concerned in all essential features ; that is to say, it must give to the resulting product the proper character and conditions. In distilleries the required amount of alcohol must be produced during fermentation, etc. It is worth while, therefore, to make a distinction between the features that are important and those that are of less importance, or of no importance at all.

In the case of *brewers' bottom-fermentation* yeast, one cannot as a rule during the early stages of fermentation, when the yeast culture is developing, judge with perfect certainty to what extent the selected race is capable of accommodating itself to given circumstances.

A small tun is needed for the first fermentations. The culture is grown for a few hours in, say, 5 litres (1 gal.) of wort at 12°–15° C. (54°–60° F.),* and then passed on into 80 litres (18 gals.) of wort at 10° C. (50° F.) placed ready in a tun that can hold about 6 hectolitres (4 brls.). Directly fermentation has set in, about 80 litres (18 gals.) of wort, of the same temperature as the fermenting wort, are added, and when the head has been formed, 2 hectolitres (1¼ brls.) of wort at the same temperature as the fermenting wort are added. When the whole contents of the tun are in a vigorous state of fermentation, the fermenting wort and yeast are all run together into an ordinary tun, and roused up with 4 to 6 hectolitres (2½

* If the culture has undergone a long journey—for instance, a long sea voyage—the first preparation should be in 5 litres (1 gal.) of wort at 20°–25° C. (68°–77° F.).

to 3¾ brls.) of wort at 10° C. (50° F.). After wort has once more been added to it, a gyle of usual length is in fermentation. This fermentation is carried through in the ordinary way. Sometimes better results are obtained if the yeast is allowed to go through a complete primary fermentation in the small tun and the resulting yeast is used for pitching a new portion of wort.

If the fermentation is allowed to run its course in the small tun, in order to subject the yeast to a preliminary test, or to enable the yeast to come to rest, if necessary, at this stage of its growth, it must, then, be borne in mind that the appearance of the fermentation will not be quite the same as in the large tuns : the head has often a different appearance, the attenuation may be greater than in the large tuns, a distinct " break " is not always seen in the liquid, taste and smell of the liquid may turn out somewhat different, and so on.

Slight differences that are perceptible in these small fermentations are, as a rule, of no importance. Only when serious variations from the rules laid down for guidance in practice are noticed, more particularly in the *smell* and *taste* of the liquid in the tun, has one cause to think that the yeast mass is seriously at fault.

When no signs of developing a " head " are visible, when the attenuation is markedly higher or lower than is desirable, or if an extraordinarily long time passes before any signs are visible of the liquid " breaking " or clarifying, then must care be exercised. If such is the case, it may be advisable simply to remove the yeast from the tun, manipulate it in the ordinary way, put again a suitable quantity of it in the *same small tun*, and repeat the fermentation to see whether the marked differences are transient and disappear or not.

It is without doubt quite possible, as experience has

shown, that the operator is often to blame for such great variations making their appearance in the fermentations in the small tuns, when he neglects to exercise the care that must be taken *to keep the yeast pure* during its growth and *to secure a proper growth* of the yeast. In some cases the irregularities that appear are not in any way attributable to the type of yeast employed, but to foreign germs, bacteria, or wild yeasts, that have crept in with faulty manipulation; in other cases he has forgotten to bear in mind that so small a fermentation must be carried through with greater care and attention than the fermentations conducted on a larger scale.

Before all things it is necessary to take great care that the pure culture, on being introduced for the first time into the ordinary wort, should at once *rapidly increase*. Arrangements must be made to ensure the production of a large number of young cells in a few hours. It is most important to attend very particularly to this when the culture is sent off to some distance in a closed can, and is perhaps many days on the journey. This is the reason why the culture is first passed into the small quantity of wort as described, and a strong fermentation is usually obtained after a couple of hours; the rejuvenated culture will then be able to make *a more rapid start* in the larger quantity of liquid, which is of the greatest importance *for the preservation of the culture in a state of purity*. The ordinary wort used in practice for developing cultures always contains foreign germs. When the culture cannot, after being sown in this ordinary wort, bring it rapidly into *pronounced* fermentation, there is every opportunity for the foreign germs to get a good start, and it is well known that these latter have the greatest power of resistance when they are able to develop at the commencement of the fermentation. It is not enough that the

yeast cells should show signs of budding a few hours after being sown in the liquid; they must rapidly bring about a strong and distinct fermentation.

There is, however, still another standing rule of equal value, but one that is often neglected. It is—to be careful of *the temperature during fermentation in the small tun.* If this point is not attended to, one not only runs the risk of the yeast being checked in its growth, and consequently the chance of foreign germs gaining a footing is made easier, but also *the character of the culture yeast itself suffers harm* in consequence. This is a fact that holds good in the case of, at any rate, a large number of yeast types, and it must never be overlooked.

The small tun, in which the pure culture is to be developed, is generally placed in the tun room, the best place for it, as it is not possible in most breweries to find space elsewhere where the temperature is suitable. As mentioned above, the fermentation is started in the small tuns at a little higher temperature than that at which fermentation is started in the large tuns. The temperature rises during a normal fermentation. When the tun room is cold, the lower temperature will have a much greater influence on the small volume than on the larger volume of liquid. Consequently during the growth of the culture the temperature will either not rise at all or else fall back. Such a mishap can be very easily avoided. Instead of using the ordinary means of isolation in the small tun that may easily carry infection if not kept scrupulously clean, all that is necessary is to raise the tun on to a couple of blocks so that it stands off the floor, and place a small spirit lamp or a candle under it. Experience has proved such a small flame to be quite sufficient to enable the yeast to bring about the necessary rise of temperature. A small cylinder may be placed round the light to keep off the draught.

There are finally about 4 hectolitres (2½ brls.) of wort undergoing fermentation in the small tun.

If the yeast should be subjected to a preliminary test at this stage, or if, on account of its specific peculiarity, it should be preferred to let it rest, the fermentation should be carried through before developing it further and the yeast settle down, as it will in the ordinary course of things. If the taste and smell differ to a marked extent from what is usual in that respect, see if these differences disappear after carrying through another fermentation in the small tun, starting the fermentation with the proper proportion of yeast.

If everything has proceeded satisfactorily, the yeast that has been grown is now added in the usual proportions to wort in one of the large tuns, and when a head has been formed, the tun is filled up either at once or gradually with wort of the same temperature as that of the wort that is fermenting.

Even if the attenuation of the first fermentation in the large tun varies somewhat from the usual limits, and this sometimes may well happen, it will not make a great difference in the end, as the beer when racked is distributed over several casks.

With regard to the so-called "break" in the liquid, it should be here pointed out that there are races of yeast that work excellently, but do not effect a true distinct break at the end of primary fermentation.

The rapid growth of the culture in the manner first described secures the advantage of its uninterrupted development; thus danger of infection is lessened, and the yeast can be transferred all the sooner to the large tuns. There are, however, a few types of yeast that suffer during the first fermentations on a large scale as the result of so rapid a growth—for example, in respect of

power of clarification—and they must consequently be allowed to carry the fermentation through to the end in the small tun.

Often the brewer does not select the pure culture from his own ordinary yeast, but allows the laboratory to select a type that is already well known in practice, giving him at the same time some hints about the best method of treating the culture.

Brewery top-fermentation yeast will always grow rapidly, owing to the higher temperatures employed, and fermentations can be started with a smaller quantity of yeast than when fermenting with bottom-fermentation yeast. It is advisable to be particularly careful when types of yeast that clarify quickly are employed, and in any case to allow the fermentation of, say, 5 to 10 hectolitres (3 to 6 brls.) to continue until a distinct clarification is noticeable before making use of the yeast produced. Only intimate acquaintance with the race of yeast itself will enable one to decide whether it will stand being rapidly developed. At any rate the development must take place at higher temperatures than in the case of bottom-fermentation yeast. No general rule can be laid down, because top fermentation is carried out at very different temperatures in different countries.

A pure culture of *distillery yeast* does not, when introduced, actually present greater difficulties in acclimatising it than ordinary yeast introduced from another distillery. The culture may be propagated in the saccharified sweet mash of the distillery, which should have a concentration of about 15 per cent. (Balling) and by addition of sulphuric acid be brought to an acidity of 0·7–0·9°. But it is preferable to employ the *Lactic Mash*. The mash must first be warmed. By this means a good deal of the foreign germs are checked, if not

completely stifled; the same effect is also brought about by the high temperature of acidification and by the lactic acid produced.

The initial quantity of mash, 5 litres, is increased to the requisite amount by successive additions, each five times as much as before.

Seeing that the acidified—lactic—mash is after acidification always warmed to 75° C. or thereabouts, it follows that the mash in which to grow the yeast culture need not be warmed again.

After the 5 litres of mash have been pitched with the yeast culture, the temperature should be about 25° C. This small portion of mash is now covered up and left to undergo fermentation in a warm place; the temperature of the mash, however, must not rise above 30° C. After the completion of the principal fermentation the acidified wort is, then, gradually increased to the requisite amount by adding fresh mash.

If the yeast developed is to be used for distilling purposes, the pitching temperature must be by slow degrees lowered until the usual level is arrived at.

As for the propagation of yeast cultures in air-yeast manufacture, a similar procedure must be adopted, only with this difference, that the mash is replaced by concentrated lactic wort (original wort). Before pitching the principal fermenting vessel, the yeast is slightly aerated.

Pure cultures of *wine yeast* are employed partly to ferment grape must, and partly to produce a normal fermentation of different kinds of fruit must.

When fermenting *grape must* with a pure culture of wine yeast, the object is to develop the selected type at the earliest stage possible, in order that it may at once hold in check the development of the many and various

germs in the must. Amongst these germs the small lemon-shaped yeast is one well-known form that commences to grow at once, and this type of yeast is commonly supposed to be a disease-producing yeast in wine. The selected race of wine yeast will therefore be enabled to assert itself more actively if introduced during the mashing or pressing of the grapes, the yeast being first strongly diluted and then poured out on them. It may be necessary to add some more yeast after the lapse of a few days.

No general rule can be laid down stating the proper proportion of yeast to add to the must. The proper proportion to add in each case depends, as already stated, upon the amount of yeast found on the grapes, which varies very much each year.

The quality of the grape must has to be taken into consideration when determining the amount of pure yeast to be used. With light wines about $\frac{1}{4}$–$\frac{1}{2}$ per cent. of vigorously fermenting must can be employed; with heavier wines up to 1 per cent.

But the importance of a proper after-fermentation also limits the amount. If too great a quantity is added at first and the primary fermentation is too strong, the yeast will work out too quickly, and the wild germs can then again take up the work at a later stage.

The proper rule would certainly appear to be not to add too much yeast, but to employ it rather sparingly.

It has been found that the proper employment of a pure culture of wine yeast at advanced stages of the fermentation is of great value—namely, where a microbiological examination shows that wild ferments are multiplying. But no general rule can be laid down even here. The matter must be settled in each particular case according to previous inquiry. All that can be said is

that it is often important to add larger quantities of fermenting grape must than stated above, up to, say, 2–3 per cent.

Pure wine yeasts have proved themselves of great use in producing a pure fermentation of the *juice of fruit and berries*.* During the spontaneous fermentation of these juices strong growths of bacteria and yeasts, having a most undesirable effect, are often the first to develop. It would therefore be advisable to pasteurise the juice before adding the pure culture yeast.

Pure cultures of wine yeast—Rhine wine yeast, port wine yeast, etc.—must not here be used in too small quantities to suppress the wild germs; and as the after-fermentation is generally of short duration, it does not matter if the primary fermentation is vigorous. A fresh culture of the yeast can be afterwards added at a later stage, if it is desired to maintain a vigorous after-fermentation continuing over a long period.

The absolutely pure culture, say 1 litre (1¾ pts.) of very diluted yeast, is added to 5 litres (1 gal.) of juice that have been previously boiled, and allowed to ferment at 20°–25° C. (68°–77° F.). When in a state of vigorous fermentation, the whole is added to, say, 1 hectolitre (½ brl.) of juice, and this juice, after fermentation has set in, is employed as a ferment in the proportion of 5 to 10 litres (1 to 2 gals.) per hectolitre (about 22 gals.). The stronger this fermentation is, the smaller will be the amount of fermenting liquid required.

Without doubt the best results are obtained by adding the yeast, after development, directly to the *mashed or pressed* fruit. The fermentations in bulk proceed most satisfactorily at 12°–15° C. (54°–60° F.).

* A methodical use of the yeasts found on the fruits themselves will often give us still better results.

(*b*) Development of a Yeast Culture in a Propagating Apparatus

Special plant has been constructed in order that large quantities of absolutely pure yeast may be ready at hand; it is so arranged that if the instructions are closely followed the pure culture introduced can be kept pure for an unlimited time. It is also necessary to provide for the stock being so preserved that it will not change its properties during continued development in these nearly closed cylinders.

If the rules of instruction are properly followed out, the first requirement can be satisfied without any difficulty. The second requirement sometimes gives rise to difficulties, and particular attention is always required in the case of special types of yeast.

Instructions in the Management of Pure-Culture Apparatus

1. When using the Hansen and Kühle model (Fig. 14) proceed as follows :—

Sterilisation

Both the cylinders and the pipes connected with them are *sterilised* with *steam*. The *air filters* are sterilised for two hours at 150° C. (300° F.).

The fermentation cylinder.—The steam is brought in through the upper tap on the pipe *kk*. All the taps connected with the cylinder are alternately opened and shut, so that the steam can pass through them all, first one and then the other, also through the glass tube *ff*. This blowing through with steam should be kept up for half an hour. The filter is screwed into place directly the steam has been shut off for the last time from the pipe with which it is connected. All the taps are closed

with the exception of *c*, and cooling commences, cold air being let in through the filter *g* and the cock *h*. The steam

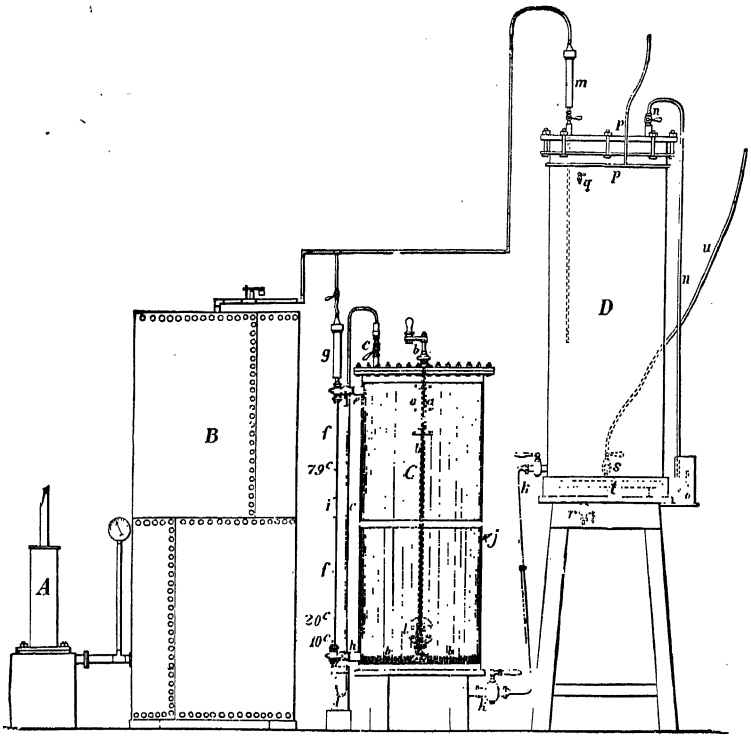


Fig. 14.—Pure yeast propagating apparatus (Hansen-Kühle).

A—Air pump. *B*—Air reservoir. *C*—Fermentation cylinder. *a*—Window. *bbb*—Rousers. *cc*—A pipe with both ends curved. *d*—A bowl containing water. *l*—Outlet tap. *ff*—Glass tube connected with the cylinder by the pipes *e* and *h*, and marked off in order that the volumes of wort contained in the cylinder can be measured. *g*—Filter. *i*—An india-rubber connection in the centre of the glass tube. *j*—Pipe and india-rubber end, through which the pure culture is passed into the cylinder. *kk*—Pipe connecting with *D*, the wort cylinder. *m*—A filter. *nn*—A tube with both ends curved. *o*—A bowl of water. *pp*—A pipe for spraying down the sides of the cylinder. *u*—Wort pipe, connecting with the cock *s*. *t*—Waste pipe for the cooling water. *q*—A tap (the cylinder is filled up to this level). *r*—An outlet pipe.

is gradually shut off, and the cylinder will be cool in about two hours' time. It is necessary to keep up a certain excess of pressure in the cylinder whilst this work is going on. In order to control this pressure with certainty, the bent end of the tube *c* is placed in a small pail of water. If there were allowed to be a vacuum for only a moment in the cylinder, impure air would be sucked in. If the steam were suddenly shut off, the resulting vacuum might be so great that the cylinder would be pressed in.

The wort cylinder.—The steam is conducted in through the lowest cock of the pipe *kk*. The pipe *su* is sterilised at the same time as the cylinder, so that the steam rushes freely through the main wort pipe that brings the wort from the hop-back. In other respects this cylinder is sterilised in exactly the same way as the fermentation cylinder. Of course this cylinder should not be cooled down.

Arrangement of the Wort Cylinder

The wort must be ready for use about ten minutes before steaming out is completed. At the same moment that steaming out is stopped, the filtered air, and afterwards the wort, which must be as hot as possible, are allowed to enter. The first small quantity of wort has become a good deal diluted with the condensed steam on flowing into the cylinder, and consequently contracted an unpleasant taste; this should be drawn off through tap *r*.

It is possible that under certain circumstances the wort may have cooled down to such an extent before entering the cylinder that suction through the tap *q* can take place. Such suction must be guarded against by allowing the cylinder to cool down to, say, 90°–85° C. (195°–185° F.) before letting the wort flow in. The pipe *u*

must in such cases be isolated. If any suction has taken place, it is necessary to boil the wort again by means of the steam coils placed in the cylinder for this purpose.

The wort flows in until it has risen to the level of the tap *q* and begins to pour out of it; the tap *s* is then closed.

The air and the steam that enter the cylinder with the wort escape out partly through *q* and partly through *n*.

Aeration and Cooling of the Wort

The tap *q* having been closed, sterile air is now passed through the hot wort, passing into the cylinder through the filter *m* and the long pipe in connection with it. Aeration is kept up for *one to two* hours before commencing to cool down the wort. Aeration is also kept up uninterruptedly whilst the wort is being cooled down.

A pressure of 1 to 2 atmospheres is sufficient in the air reservoir. There should always be a slight excess of pressure in the wort cylinder to ensure the wort taking up sufficient oxygen, and no unfiltered air being drawn in.

After one or two hours' aeration of the wort, the cylinder is cooled down, water being sprayed down the sides, outside, through the ring *p*, until the temperature of the wort is reduced to about 10° C. (50° F.) in the case of bottom fermentation. This occupies about one hour. Any further cooling, if necessary, must be carried on with ice-water. After cooling down to, say, 11° C. (52° F.), froth passes through the pipe. It is not advisable to aerate too vigorously, as too much wort may be lost in so doing. The froth is less troublesome if the receptacle *o* is filled with warm water. In case of top fermentation the wort is cooled to, say, 20° C.

Transfer of the Wort to the Fermenting Cylinder

The long pipe from the filter is closed, and the short pipe opened. The cock *n* is closed, and then the cocks on the pipe *kk* are opened, and the necessary amount of wort is allowed to flow in.

If, as in the drawing, the feed pipe *j* is situated on the side of the cylinder, the pure yeast must be passed in before the wort comes up to its level. In some cylinders this pipe is placed in the top of the cylinder. This arrangement has the advantage that the operator introducing the pure culture can place the lamp in front of him, and complete the work without further assistance, whereas in the first case the assistance of another man is required to hold the lamp.

The culture is passed into the cylinder from a Pasteur flask or from a travelling can* (Fig. 15), constructed on the same principle as the Pasteur flasks, but with the addition of a cotton-wool filter to the pipe through which the air is drawn in whilst the yeast is being poured out. The can is washed with alcohol, and shaken for a few minutes to loosen the yeast and distribute it through the water in the can, and the side-tube of the can or flask is connected up, in a small flame, with the small rubber connection on the side or the top of the fermenting cylinder. This rubber connection is closed with a glass stopper. A spring clip is fixed on the rubber, or this is fixed on an ordinary tap. After the pipe on the side of the can has been connected with the cylinder, open first the tap on the cylinder and then the tap on the side pipe of the can; a large proportion of the contents of the can will be at once discharged into the cylinder owing to the pressure of the

* This holds, say, 1 litre, and receives the yeast grown in a number of flasks.

carbonic acid gas : then only is the tap situated on the other pipe connected with the can and below the cotton-wool filter opened, and the remainder of the yeast allowed to flow out of the can into the cylinder, or forced out by blowing through the filter. The spring clip or the tap on

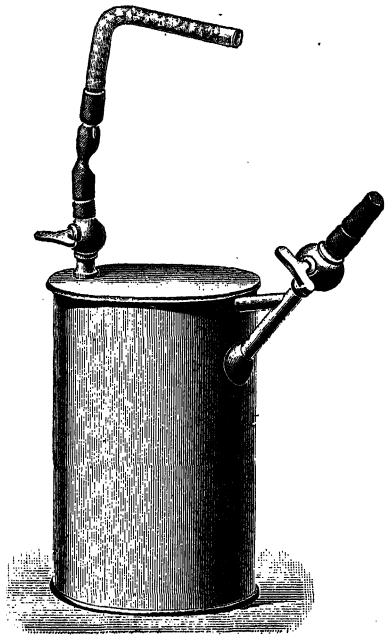


Fig. 15.—Travelling can for the despatch of pure cultures of yeast.

the cylinder is then closed, the glass stopper is heated in a flame, the side pipe of the can is withdrawn from the rubber tube of the cylinder, and the latter is closed with the glass stopper whilst still hot. The glass stopper is taken out of the rubber tube, at the same time squeezing the tube in order to drive out any liquid remaining in it, again heated, and finally fixed tightly into the rubber tube again.

It is advisable for breweries that obtain their cultures from a distance not to fill their cylinders full at once, but only to run in about 15 litres (3 gals.), and let the yeast develop in that first, at a somewhat higher temperature, say 15°–20° C., and when a vigorous fermentation has set in to add what wort is still required to fill them.

The *withdrawal of the yeast* from the large size cylinder is usually carried out as follows: The fermented liquid is first withdrawn under slight pressure; a little cooled and aerated wort is then run in from the wort cylinder, and yeast and wort roused up with the rouser in the fermenting cylinder and the diluted yeast then drawn off. Some of the yeast is left behind to start a fresh fermentation. What amount is necessary must be ascertained, as it may vary with the type of yeast. The following may serve as a guide:

The gauge tube or the cylinder is marked off with three marks at 80, 20, and 10 cm. (32, 8, 4 inches) from the bottom of the cylinder respectively. When the fermentation is completed, the beer is drawn off under pressure through the tap *l* until frothing commences. Wort is then run in from the wort cylinder until the next lowest mark is reached, roused up, and drawn off into a clean tub until the lowest mark is reached; then wort is again introduced, until the next lowest is again reached, roused up, and again drawn off until the lowest mark is reached. The new fermentation is started with the yeast now left behind, wort being run in up to the top mark on the gauge tube.

Fig. 16 is a photograph of the present type of Hansen-Kühle Apparatus, as constructed by the firm W. E. Jensen & Son, Copenhagen.

If possible, the brewery wort must be used in the cylinders in the state it is in as it passes from the hop-back to the coolers, being carried to the cylinders by a

pipe connected with the main wort pipes delivering the wort on to the coolers from the hop-back.

Similar propagating apparatuses have been constructed by Bergh & Jörgensen, Brown & Morris, Lindner, Thausing and several others, but none of them is more practical or less complicated than the Hansen-Kühle apparatus.

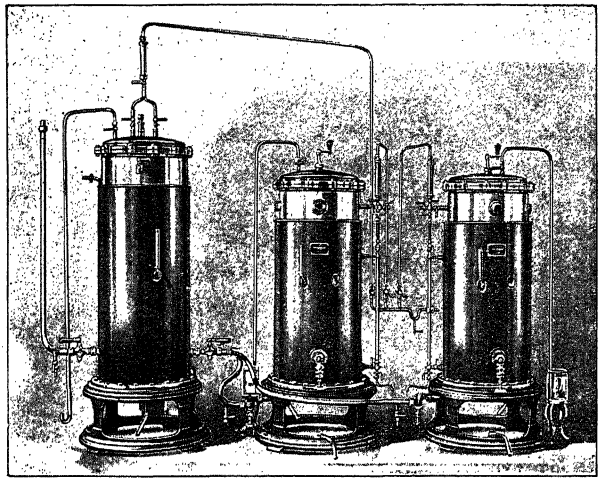


Fig. 16.—Hansen-Kühle apparatus.

As the price of the apparatus, however, is rather high and it requires constant and careful tending, several brewers, distillers and wine manufacturers prefer to develop the pure culture in a tun as already described. But in order to do this in a more safe way, with less risk of contamination, there has also been constructed apparatuses for this purpose, for instance the Stockhausen-Coblitz Apparatus and the " Aigel " Apparatus, both of which are sold at comparatively low prices.

We shall here give a short description of the " Aigel " apparatus, Fig. 17. The apparatus consists of three

vessels made of copper or stainless steel. In case liquid pure cultures are used, only vessels I and II are required; for the propagations of dry cultures all of them are

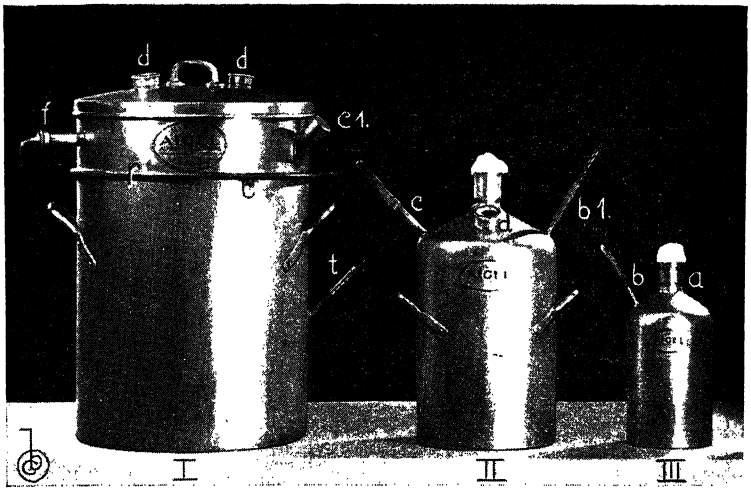


Fig. 17.—" Aigel " apparatus.

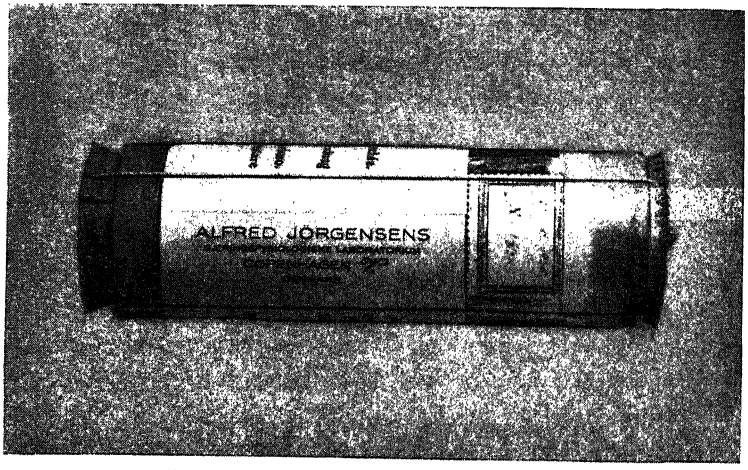


Fig. 18.—Dry culture, ready for shipment.

employed. Before being used the vessels are carefully cleaned and left to stand overnight, with a small quantity of alcohol on the bottom of each.

If a dry culture is to be developed, about 1 litre of tap water is boiled for 20 minutes in vessel III and allowed to cool to room temperature. The culture (K) is placed on top of the vessel, the cotton plug having been removed; the contents are then pushed down and the cotton plug replaced. The vessel with the yeast remains at room temperature for a couple of hours, being shaken now and again. In the meantime vessel II has been filled with about 5 litres wort. This is taken from the hop strainer, connecting the rubber *c* with a small cock placed on the hop strainer. When the wort has been cooled down to about 25° C. (77° F.) the yeast is transferred to vessel II by connecting the rubber *b* on III with b_1 on II in the flame of a Bunsen burner. Through the windows, *d*, the liquid can now be observed and as soon as the Kräusen are seen, the contents are transferred to vessel I. Vessel I has also been filled with boiling hot wort from the hop strainer; in this case it can be done by removing the cover. The wort is cooled down to about 22° C. (71° F.) by running cold water through the sprinkling ring, placed on the outside of the vessel, and at the same time aerating it by introducing air through the filter *f*. A thermometer, *t*, indicates the temperature of the wort. The fermenting liquid is introduced into vessel I by *c*. When fermentation has started, the whole contents of vessel I are transferred to a small tun containing about 1 hl., wort ($\frac{1}{2}$ brl.). If liquid cultures are employed, the metal flask for the transmission of pure yeast cultures is connected with b_1 of vessel II.

It is known that if the wort is re-boiled in the cylinder, a considerable amount of matter is thrown out of

solution, thus giving it a new composition, and there is no doubt that if the yeast is grown for a length of time in such wort, different from the wort used in the brewery, its character may be altered little by little. When circumstances render it necessary to boil the wort in the cylinder, one often is obliged to introduce fresh cultures of yeast at intervals of a few months.

The hot wort in the cylinder must be cooled down slowly, and must be well aerated both at high and at low temperatures. When the selected race of yeast appears to work irregularly in any way, and particularly if clarification proceeds too slowly, experiments should be carefully carried out in different directions to ascertain the cause, the length of time already given for cooling being taken as a guide. One valuable remedy to be recommended in such cases is to prolong the length of time of aeration at the higher temperatures by running hot water into the jacket surrounding the cylinder.

It is, however, as a rule easier to master the clarification and attenuation when close attention is paid to *the fermentation itself*. Not only *the amount of yeast* used, but *also the temperature, duration of the fermentation*, and *aeration* during fermentation, all play a part when a good yeast is to be continually produced in the cylinder.

Fermentation proceeds under different conditions in the cylinder from those which exist in the open vessels, and one cannot therefore draw a very close comparison between the two classes of fermentations.

It is necessary to commence with a suitable quantity of yeast in order to develop the required quantity within a reasonable time under ordinary conditions of fermentation. It would hardly be correct to set up exactly the same proportions as in the large tuns, in order that the *course of the fermentation* should be the same, because the carbon

dioxide given off during the fermentation can only escape through the very narrow bent tube, so there will naturally be pressure on the surface of the liquid, even when the carbon dioxide is removed by air being pumped in; consequently the fermentation will proceed more slowly. Therefore some yeast types at any rate require to be used in rather larger quantities than in the ordinary tuns.

Temperature, however, is of more importance. It is certainly inadvisable to work continually at temperatures that differ considerably from those employed in practice. Higher temperatures may be used experimentally for certain exceptional fermentations. Cases have arisen in practice where it has been advantageous to do this: for example, a brewery top-fermentation yeast, cultivated *once* at a temperature only just lower than the temperature in which it could live, gave a better clarification than before. On the other hand, it is not at all advisable to let the yeast in the cylinder be acclimatised to temperatures quite different from those maintained in the tuns. The fermentation should be carried on one or two degrees higher than in the tuns; cooling down can be carried out at the close of the fermentation just as well in the case of top yeasts as of bottom yeasts, in order to ensure a better settling out of the yeast.

The *duration* of the fermentation also plays a part. To start with, the rule must be laid down that the yeast in the cylinders must pass through a *complete primary fermentation*. To take the yeast when a vigorous fermentation has set in—that is to say, when the yeast is still suspended in the fermenting liquid—certainly offers the advantage that the yeast can be renewed in the large tuns more frequently; but to carry out this principle as a regular thing may be detrimental to the particular type. Normal working is to take the yeast after it has settled out

of solution on the surface or at the bottom: to take it when fermentation is at its height is an exception to this rule.

Aeration in the fermentation cylinder is of the greatest importance. The view has been expressed that as the yeast in the closed-in cylinder is cut off from the influence of the air, fermentation must be assisted by pumping air through the liquid. But experience has shown that in this respect many yeast types, both top and bottom yeasts, cannot stand such vigorous aeration, and perhaps particularly the strong motion set up; if so treated, such yeasts may undergo a change both in respect to clarification and also powers of attenuation. The rule as regards aeration in the fermentation cylinder must, under ordinary circumstances, be as follows: Immediately the fermentation is started, a slight current of air is passed through the liquid, partly to secure a thorough distribution of the yeast cells, partly to replace the free oxygen taken up by the cells during the first hours. The bottom air valve is then closed, and the liquid is allowed to remain undisturbed. Subsequently during the whole course of the fermentation a very slight current of air is passed *over the surface* of the liquid to drive off gradually the carbon dioxide produced during fermentation.

The afore-mentioned rules are given as *general directions* for the proper working of a pure yeast culture apparatus. If any change appears in its quality, the special requirements of the type of yeast in use must be carefully studied and its treatment in the cylinder regulated accordingly.

The pure-culture yeast cylinders have been found useful not only in breweries but also in *distilleries working with molasses*, and in other *distilleries and yeast factories*. No difficulty is found in the molass distilleries in working the cylinders under the same conditions as those of the large

fermentations, as the liquid employed does not appear to undergo any important change in respect of its nutritive value for the yeast when it is boiled in the cylinder. A sufficient supply of nitrogenous compounds is sometimes added to the molasses in the cylinder to secure a more vigorous growth of the yeast. On the other hand, in ordinary distilleries and yeast factories the liquid undergoes considerable changes owing to its being boiled to secure sterilisation—a considerable quantity of nutritive matter being thereby thrown out of solution. This loss must be made good, but it still is not possible to restore to the liquid the same composition that it usually has. If the yeast undergoes too great a change under these conditions, there is nothing for it but to pass it several times through a lactic acid mash before it is used on a large scale.

The normal way of withdrawing samples from the cylinder for analysis is through the pipe by which the pure culture is introduced. A Pasteur flask or a flask specially constructed for the purpose of forwarding yeast in it (Fig. 9, left) is connected up with the cylinder in the same way as flasks containing pure cultures are connected. The sample is taken a few days before the close of the fermentation. The greatest possible care must be taken in collecting these samples, as the most searching methods of analysis are employed to ascertain if the culture in the cylinder is absolutely pure. It sometimes happens that slight traces of the liquid have been left behind round the mouth of the pipe or the rubber tube on the cylinder. In order to be quite safe, the tube and stopper are first washed with a little alcohol; the glass stopper is then heated in the flame and replaced in the rubber tube and allowed to remain there until quite cool, and then the sample is taken.

A portion of the sample is examined to detect wild

yeasts, Torula and Mycoderma, and a portion to detect bacteria.

In order to detect the slightest trace of wild yeast, a portion of the sample is placed in a 10 per cent. solution of cane sugar, with tartaric acid (1–4 per cent.) added. An average sample of the yeast developed in this solution is taken, and again treated in the same manner. A little of the growth is then passed into a flask containing hopped wort, and re-invigorated. The least trace of wild yeast or Mycoderma originally present will have become so strongly developed by this treatment that the infection will be at once evident on examining spore cultures of the growth developed on blocks of gypsum.

The rest of the sample is next examined in order to detect bacteria. If actual bacterial *development* has taken place, the microscope will reveal it. In order to discover even a *trace* of bacteria capable of developing, a few drops of the liquid are introduced into a flask containing neutral or slightly alkaline yeast water, and kept at 25°–35° C. (77°–95° F.). The bacteria will then develop in this solution. Not all bacteria will, however, when grown in this way, make the liquid cloudy; after allowing sufficient time for their development, the contents of the flask must therefore be examined under the microscope.

A general microscopical examination of the yeast in the cylinder is undertaken to find out whether the yeast has grown *uniformly*. It sometimes happens that the inside tinning of the cylinder wears away in places, the liquid takes up salts of copper, and consequently the growth of the yeast is sickly. This is ascertained by a microscopical examination, because a proportionately larger number of exhausted cells will be found—that is, cells whose contents have a very different appearance from that of normal cells.

It is important to empty out the fermentation cylinder at least once a year, to examine its interior carefully and inspect all the smaller parts of the apparatus.

III. DEGENERATION OF THE YEAST

Varieties Developed whilst Yeast is in Use

The pure cultivated alcohol-yeast is not only free from all disease-producing germs, but consists of *one single species* only.

Only when employing one single species is it possible, as already stated, to carry on the fermentation with regularity and certainty. Every attempt to secure constant and uniform results will be wrecked if the yeast is a mixture of several different types, because such a mixture cannot preserve its original composition unchanged after repeated use.

Only when the yeast is composed entirely of one species is it possible to obtain a real knowledge of its nature.

In practice it is clearly impossible to ascertain by a *systematic selection* the yeast most suitable for one particular method of work, when in every fermentation more than one type is to be found.

But practical work with *pure cultures* of yeast has shown that *there are very great race differences* between the cells of the same yeast *type* in daily use. In the days when unpurified yeast was used, there was no idea that such was the case, and that these differences might be turned to great advantage.

Accurate conclusions as to the great difference between yeast *types*, particularly with regard to the effect of their respective characters in practice, can only be drawn after carrying out *accurate comparative experiments* in a laboratory

especially fitted out for the work in view.* In carrying out such accurate comparative experiments new discoveries may be made and marked industrial advances consequently secured.

When using the different types of yeast in breweries, distilleries, the wine industry, etc., it must be clearly understood that *there are definite limits to the possibility of a particular type bringing all its qualities to the front under industrial conditions.*

Certain characteristics of yeast may make their influence felt in varying degree under very different conditions in practical life; whilst there are others that only exert their influence when certain definite external conditions exist.

To take an example of the first state of affairs. A yeast that imparts a particular pronounced flavour in hopped wort, or in wine must, will always impart the same flavour to the same type of liquid, even when the composition of the latter is changed not a little. The *strength*, however, in which this flavour is present will vary with the composition of the liquid and with the whole course of the fermentation.

Yeast types which set up a vigorous alcoholic fermentation are so able, even when ordinary wort or when must shows varying compositions, to make their influence felt that the final attenuation shows the character of the race. But not only the real limit of attenuation, but also the stage when the fermentation is seen to be working particularly vigorously when compared with that produced by other types, can vary in the case of the same type, because they depend upon outside conditions.

As an example of the last-named characteristic, it may be said that weak fermenting brewery yeasts often only show this characteristic feature when the wort has a

* Refer to the section dealing with the production of pure yeast.

composition favourable to it and the fermentation is conducted in a particular manner.

Every yeast, even the most typical, is accordingly to a certain extent dependent upon the conditions under which work is carried on. The result of the fermentation is a product of the yeast and liquid; a selection must therefore be made between the types and also *between the varieties of the same type* in order to select the particular race that will secure the desired result *under the particular circumstances that present themselves.*

When this has been found, it is noticed that the slight variations that make their appearance in every method of work have no influence on the result of the fermentation as a whole. In other words, under so-called normal conditions of work, the selected type of yeast will work normally in respect of the essential features. It is actually in this respect that the great advance brought about by the employment of pure yeast lies.

But, just as the yeast is always dependent upon the nutritive liquid and external conditions, so also the *long-continued influences of any particular kind* will be apt to cause radical changes in the character of the yeast race, and it may under such circumstances almost entirely lose, for example, qualities that are appreciated in practice. Such influences may, for instance, be introduced into a brewery, when new plant is put up; when the composition of the water is changed; when the aeration of the wort is conducted in a different way, and so on. The fact that under such conditions changes in the character of the yeast mass itself can actually take place is borne out by special experiments made in practice, and, consequently, the unsatisfactory issue of fermentation is not attributable solely to the altered composition of the fermented liquid.

When ordinary unpurified brewery yeast was used,

one was accustomed to the fact that the fermentations showed variations during their course, and one came to look upon it as quite unavoidable. But the use of selected races of yeast has led to more rational methods of work and the demands for uniformity in fermentations have increased.

If the yeast mass has manifestly changed its character, find out first of all if wild yeasts or bacteria are developing.

Only when the yeast is found to be sufficiently pure for commercial purposes are such deviations to be accounted for by a *degeneration of the yeast* itself. This is only natural; but the difference deserves to be brought to notice, because the two things are often confused one with the other. It must here be borne in mind that a pure primary fermentation is no absolute security for the beer being pure at the end of the after-fermentation. Practice has shown often enough that it is necessary to control the after-fermentation also, because certain impurities which are found in the casks are able to exert an influence in the last stages of the fermentation.

The degeneration of the yeast manifests itself, according to the author's observations, by the fermented liquid developing a different *flavour* or the *attenuation* or *clarification* being different from the normal state of affairs.

The comprehensive researches carried out in the author's laboratory in regard to the yeast used in daily practice have disclosed the important fact that it is *not all the cells that degenerate* in such a case. If a sample of this pure yeast is taken and a dilution made, the individual cells being well separated from one another, a comparative series of fermentations will show that there are differences between these cells. Some of the new growths will show the newly developed disagreeable character to a marked degree, others to a less extent; but it has always been possible

on taking a sample from the tun to find cells of the same race that had not changed their character.

Based on the foregoing remarks is the following method of regenerating pure yeast. Single cell cultures are prepared from it as described in a previous section, and a selection is then made by means of comparative fermentation experiments among the isolated single cell cultures to obtain a culture similar to the original. This culture therefore starts from a particularly resistant cell.

The pure cultures should be preserved in the laboratory in the manner described in the following section. Therefore when the yeast degenerates, one has first to fall back upon the culture preserved in the laboratory, and develop a pure supply of yeast from it for practical use. In this manner the yeast is more quickly regenerated. The method first described solves the difficulty if the yeast preserved in the flasks has undergone any change.

The foregoing statement that some cells, having varying characteristics, will in the course of time appear in the yeast as a consequence of the changed composition of the nutritive liquid, is not to be confused with that change in the attenuation which may *suddenly* make its appearance when a malt of very different composition from that previously used is taken into use. When this happens, and the usual general known rules do not help, one must, for a time at any rate, use a yeast of a different type.

It is evident that the afore-mentioned system of selection between individual cells can be utilised to *improve still further*, in certain respects, a yeast that is giving satisfaction. Yeast samples can be taken from fermentations in the factory that show the desired characteristics to a marked extent, and from this yeast pure cultures may be systematically developed. As an example, it may be mentioned that brewery bottom yeasts which develop

a very strong fermentation, as a rule clarify out slowly and with difficulty. In this case the object of a *systematic selection* has been to separate out the individual cells that will produce the strong fermentation characteristic of the type, but will at the same time clarify out with less difficulty during after-fermentation.

IV. PRESERVATION OF YEAST

Many races of yeast may be preserved in the laboratory for a longer or shorter time without changing their specific qualities, which make them valuable in practice. One can therefore always renovate such a yeast by growing a very little of the culture that is being preserved, and gradually increasing the quantity by passing the young growth to larger and larger flasks.

The best-known medium for preserving yeast is a clear solution of ordinary commercial cane sugar and distilled water. The solution—10 per cent.—is filtered bright and sterilised in the flasks. The flask used for these cultures is one designed by the author; it takes up less room than the Pasteur flask, and evaporation of the liquid during long storage is checked (Fig. 9, right).

A small quantity of the selected culture is introduced into the cane sugar solution, and the flasks are kept in a dry place. If the cotton-wool filter in the cap becomes damp and consequently mouldy, mould and bacteria can easily grow through it and infect the culture.

The culture must be kept in the dark and undisturbed. If it is often shaken up it will be weakened, and may change its character.

With careful attention many brewery, distillery, and wine yeasts can be preserved in the foregoing manner. There are exceptions among top-fermentation brewery yeasts, for example, when it sometimes happens that after

about six months' preservation changes have set in in the character of the race; consequently one has to fall back upon the yeast used in the brewery in order to secure a normal culture. Also a few of the bottom-fermentation yeasts, that ferment only slightly, undergo changes after being kept for a comparatively short time.

Variations in temperature that are not too marked do not seem to have any effect on this method of yeast preservation; it is, however, clearly impossible to lay down any general rule that is suitable for so many widely different races.

When the culture is required for use, the stock flask is connected on to a Pasteur flask containing the liquid, well sterilised and aerated, that is used in practice. The flask with the cane sugar solution in it is shaken most carefully, only just enough to render the liquid slightly clouded. A few drops are then transferred to the Pasteur flask. The first cultures are allowed to proceed quietly, the fermentation being allowed to finish before passing the yeast to a fresh flask. Only when one has ascertained that the dormant yeast will stand being rapidly developed can one push on the propagation after the yeast has shown the first signs of fermentation in the flask.

In practice, distillery yeast is preserved in a pressed state. Brewery yeast may also be preserved in the same way, if it is desired to keep the yeast when no brewing is going on, in order to commence a new season with the same yeast, and use it until a pure culture has been worked up. After the yeast has been washed, it is pressed in double-fold or three-fold bags and hung up in a dry, airy place. If any mould appears on the outer bag, this is taken away and the inner bag is washed with alcohol that contains a slight trace of salicylic acid, or with a solution of water and boracic acid. The washed yeast may also be pressed and

stored in newly pitched or perfectly clean barrels, which are then closed and stored in ice.

It has often been found more suitable to preserve the brewery yeast in a liquid state. The yeast can be washed several times in water that has been well boiled and then aerated. The *thin solution* of yeast is then poured into a cask that has been freshly pitched; the cask is then tightly bunged down and covered over with ice. Or the yeast can be passed into the cask after being *simply strained off*. A yeast carefully handled in this way has been known to keep all right for two months. It naturally follows that some of the cells have died; therefore, when using the yeast again for the first time, a larger quantity than usual should be used; also, the fermentation should be pitched at a somewhat higher temperature. The visible phenomena of the fermentation will be rather different the first time from what they usually are, and the course of the fermentation will also proceed somewhat differently. This yeast is only used, however, a few times until the new culture has been worked up.

Hardly sufficient emphasis can be laid upon the fact that such methods of work are very much to be preferred to the introduction of a *strange unknown pitching yeast*, that may in one moment introduce an infection into the brewery, or distillery, whose removal may only be accomplished after many months' effort.

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